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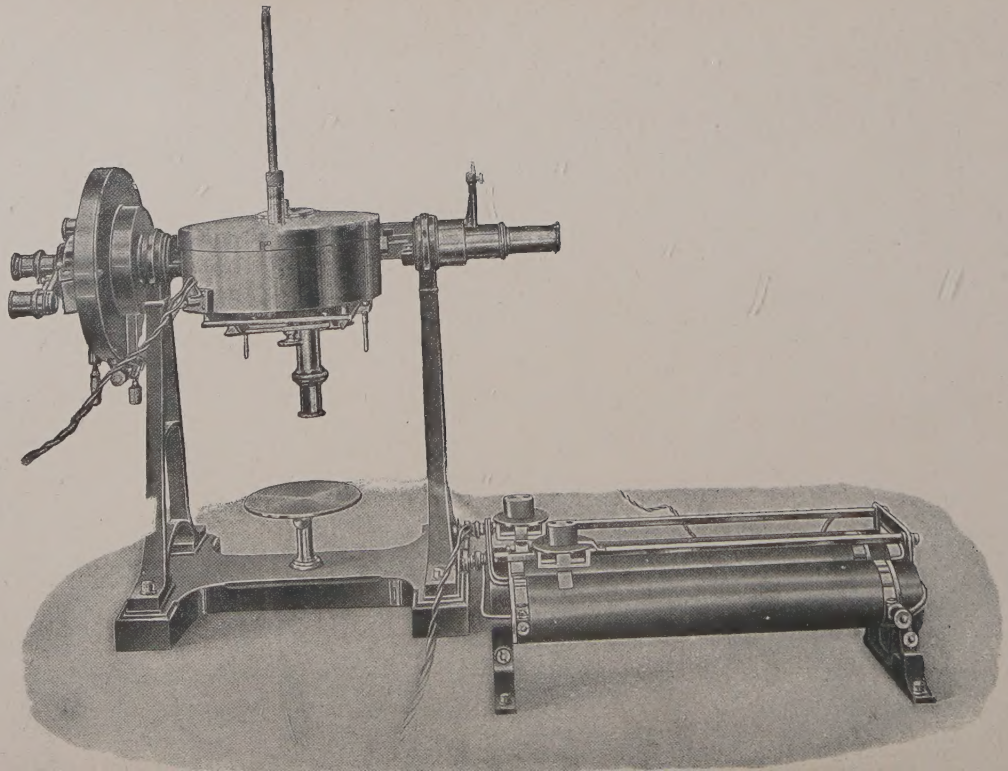

DEFENSIVE FERMENTS OF THE ANIMAL ORGANISM

against substances out of harmony with the body, the blood-plasma and the cells; their demonstration, and their diagnostic significance for testing the functions of different organs

BY

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With 11 Text Figures and one Plate

THIRD ENLARGED EDITION

ENGLISH TRANSLATION BY

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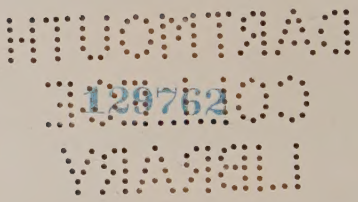

TO

MY FAITHFUL COLLABORATORS.

Preface to the English Edition.

It is more than three years since Abderhalden first published his results in regard to the sero-diagnosis of pregnancy. In the course of his general studies on the nature and properties of the ferments in the blood, and on their relations to metabolism, he came across an instance of their specific action, which suggested the possibility of diagnosing the condition of pregnancy by their means. And it was on the basis of the same methods as had been employed years before by himself and his followers in their preliminary theoretical investigations, that he was led to the great discovery of the demonstration of specific ferments in the blood-serum; that is to say, by the use of the optical method and of the dialysation process.

In view of the possibility of the practical application, in medicine, of these new methods of research, for the purpose of making differential diagnoses and of testing the functions of organs in various diseases, they have been taken up by many members of the medical profession. They were first employed by the gynæcologist; but there is now hardly any branch of medicine left in which the application of these new methods has not been attempted, and in the course

of time interest in these new weapons of research has rapidly increased. Much has been done, for the popularization of these methods, by the kindness which has been shown by Abderhalden and his assistants to all those who were willing to acquaint themselves with the rather complicated technique involved in them. He found room in his institute for all who wanted to come; every written inquiry was promptly answered; and reagents, such as placenta-albumen and peptone, in the preparation of which some difficulty is met with, were freely supplied from his laboratory.

The conception of "harmony and disharmony" has been employed by us, in order to represent the meaning we attach to Abderhalden's terms "fremd" and "eigen." These phrases, though they have been translated literally by some, do not seem to us to be amenable to direct translation.

In presenting this translation of the latest edition of Abderhalden's work on defensive ferments, I have been inspired by the hope of being able to excite or further, in regard to this important line of modern research, the interest of many to whom the German text may be inaccessible.

J. O. Gavronsky.

7, Cambridge Terrace,
Regent's Park.
February 23, 1914.

Preface to the Third Edition.

It took less than three months for the Second Edition to be exhausted, a pleasing sign that this new field of research has excited much interest. The number of works which have been completed on the basis of the principles there laid down, and of the methods there disclosed, exceeds one hundred and twenty! Every week brings forth new works. I am not sure whether that ought to give me entirely unbroken satisfaction. The fundamental works, which have arrived at a definite conclusion in regard to the elaboration of the dialysation process and of the optical method, have been produced during the last twelve years or so. The "theoretical" part, which pointed to the possibility of a sero-diagnosis of the functions of organs, was practically established six years ago. Experiments on animals were started on a large scale, so as to allow for all possibilities. Over and over again doubts cropped up which had to be settled. The astonishing result was found that, in disturbances of certain organs, only their albuminous constituents suffer decomposition. These discoveries were not made public, and only those results, which were established in investigations on

pregnancy, were published. Pregnancy is a condition which allows of no misinterpretation. In almost every case the clinical diagnosis can be compared, with absolute certainty, with the result of the serological diagnosis. The actual diagnosis either corresponds with the former, or it does not. These clear conditions, however, are not presented by the other morbid processes. A certain disease may be accompanied by all kinds of other disturbances of the functions of the organ. Very seldom are we faced with the presence of "pure" disease. Therefore, we are bound to conclude that only the worker in a hospital is in a position to judge, to what extent serological investigations can be applied for testing the functions of an organ. In this case two aims have to be distinguished. The serological diagnosis can, in many cases, widen our understanding of the disturbances occurring in a given disease. We gain an insight into long suspected functional troubles of certain organs, or discover that others, which had never been thought of, regularly produce disturbances in a certain disease. It is an entirely different question to ask whether the serological diagnosis of an organ can be applied to differential diagnosis, *i.e.*, whether we are entitled to accord a preference to this, as against any other, method.

Many years may be required before the question of the practical value of the methods worked out can

be decided for each separate case. Every research, which has not been carried out with absolutely unobjectionable technique, delays our arrival at a clear appreciation of the suitability of the methods. There are practically no methods which, on first acquaintance, will lead anyone to good results. Often weeks have to be spent in preliminary studies, before facts are acquired which entitle us to apply the required methods to certain questions. No conscientious student would publish these preliminary studies, but would treat them as exercises. Owing to a very extensive experience of my own, I cannot deny that many preliminary studies of this kind have been published. It is only work that is deliberate, and that is based on a complete command of methods, that can lead to satisfactory results. It is, besides, the duty of the clinical worker to thoroughly study each case, and to follow it out to the end.

As yet, it is too early to criticize the works that have appeared, and I have contented myself merely with summarizing such as have come to my notice. Then the results of recent experimental researches have been referred to. The question of the specificity of the substrates is discussed, and, finally, in the description of the technique, some recent experiences have been considered.

EMIL ABDERHALDEN.

November, 1913.

Preface to the Second Edition.

THOUGH scarcely a year has elapsed since the publication of the First Edition, yet it has been possible to widen the scope of the Second in many points. Since then, numerous investigations in different fields have been either begun or completed. The most important results of these investigations are given at the end of this little volume.

The term "protective ferment" has been dropped, because it may easily convey the idea that these ferments, which are called into action by the presence of substances out of harmony with the plasma, are unconditionally protective. The term "defensive ferment" will more readily suggest the notion, that the animal organism attempts to defend itself. By means of decomposition it often deprives disharmonious substances of their specific character, but in many cases the defensive ferments form decomposition stages which are more dangerous than the substrates they attack.

May this new edition find the same friendly welcome as the first.

EMIL ABDERHALDEN.

Halle a/S.,
June 15, 1913.

Preface to the First Edition.

In my text-book on Physiological Chemistry, published in 1906, I made an attempt to harmonize the defensive measures, adopted by the animal organism against products generated by cells out of harmony with the body, with the metabolic processes of the individual cells of the body. I was of the opinion that, when an invasion takes place of cells which are out of harmony with the body, the blood or plasma, and the cells, the cells of the body respond with counter-measures which are not entirely new to the cells of the particular organ or of the blood; on the contrary, I tried to bring the whole question of the so-called reactions of immunity into close line with processes that are normal, and consequently familiar, to the cells. From the point of view stated in the above-mentioned text-book, I attacked experimentally the problem of the method of defence, used by the animal organism, against the invasion of substances out of harmony with the body, the blood plasma, and the cells. In the first place I studied the question whether normal blood plasma contains definite ferments; and, in the second place, whether the introduction of disharmonious substances is followed by the appearance of ferments which were not there before. I found, in fact, that, after the

introduction of substances out of harmony with the blood plasma, ferments appeared which are capable of transforming these products, and of depriving them thus of their specific character. These facts established beyond doubt one means of defence possessed by the animal organism against the invasion of disharmonious substances.

My thoughts then turned at once to the relation of these facts to immunity, and especially also to anaphylaxy, and I undertook experiments to decide the question as to whether the animal organism develops any ferments of a specific nature against substances produced by micro-organisms. And I was particularly interested in the question whether the stages that arise, in any given case, during the decomposition of a particular substrate vary with the species of the invading cell, and whether this may not give us the explanation of many phenomena that appear in the course of certain infections. Finally, I was able to demonstrate that, during pregnancy also, the organism defends itself, by means of ferments, against certain constituents which are passed into the blood, most probably from the cells of the chorionic villi, and which, though in harmony with the species, are out of harmony with the plasma. This observation renders possible a diagnosis of pregnancy.

The above statements have a bearing on a great

number of particular problems connected with immunity, which still await solution; nor is there any doubt that many well-ascertained facts are closely connected with the results of our researches. Even now it would be tempting to select suitable instances from the mass of my particular observations, with a view to giving a more general signification to the views I have formulated on the means of defence possessed by the organism against the invasion of substances or cells that are out of harmony with the body. For the time being I have refrained from doing so, as the mere enumeration of closely related observations, quite apart from a discussion of all the hypotheses put forward, would enormously increase the scope of this little volume, and incidentally would interfere with a clear insight into our subject.

Again, it is very difficult, for those not actually engaged in research work on immunity, to keep in touch with all the communications made at different times concerning ideas and theories that are constantly changing, and above all to find a sure footing amongst the somewhat pleonastic terminology and nomenclature employed. Theory and actual fact form, in this field of research, a closely interwoven net of conceptions; so much so, that only those, who have already acquired, by actual corroboration, a thorough knowledge of all the problems connected with the subject, are able to trace sharp limits

between hypotheses and facts. For these reasons I have limited myself to making mention of those works which either are closely connected with my own researches, or else will be of special service to the reader, in that the full lists of references they contain will be a guide to further study in this field of research. This limitation alone has enabled me to present a picture—which, I hope, is quite clear—of the development of my own investigations, and to show how I arrived at the doctrine of the active part played by ferments in connection with disharmonious substances.

The comprehensive survey, which I now present, has resulted from the fact that many problems have been so far advanced, recently, by means of experimental work, that it seemed advisable to take stock of the observations that lie stored in numerous publications. And, on the other hand, I find that the further study of particular problems can be carried on only in institutions supplying means and apparatus which I cannot command. One man by himself is able in certain problems to reach only a certain point. He takes over, as it were, an edifice which has been built up to a certain height from all possible sides. He tests the scaffolding—the existing working theories—to see whether it will last any longer, or whether it must be replaced; and, more important than that, decides

whether the structure itself is perfectly sound. He then builds further, but in most cases makes only a tiny addition. It is very easy for a single observer to lose a clear view of the whole, through using too complicated a scaffolding. Others follow; they test the solidity of the structure, they move the misplaced bricks into their correct position, and give a finishing touch to the parts that are insufficiently trimmed. Each new workman brings new tools, new ideas, and his own extensive experience with him, and tackles the whole structure from different points of view. Then the scaffolding is removed, and a mighty building appears, which scarcely gives any idea how diverse were the plans on which it was founded. So, too, this contribution to our knowledge of the functions of the cells may be considered only as an attempt to adjust a new stone in the already existing structure, and to construct a scaffold from which further progress may be made.

In conclusion, may I be permitted to express my heartiest thanks to my collaborators, whose untiring energy has made it possible to accomplish so many single experiments in such a relatively short time, and to work out different problems from various standpoints at the same time.

EMIL ABDERHALDEN.

Halle a/S.,
April 15, 1912.

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Defensive Ferments of the Animal Organism.

THE question has frequently been raised, whether unicellular organisms exhibit simpler processes, in their general organization and metabolism, than do organisms composed of numerous cells. A priori, it is conceivable that organisms of a morphologically simpler construction are composed of simpler combinations, and that their metabolic processes follow a simpler path, than is the case in those forms of life in which the body is built up by the co-operation of different cells. But all our experience, hitherto, has proved, that even those cells which are constructed on a simple plan morphologically do, when studied from a purely chemical point of view, show exceedingly complicated relations. Indeed, the study of the processes of metabolism in unicellular forms of life is a study all the more difficult, in comparison with that of more complicated organisms, in that, in the former, it is so difficult to separate the actually

absorbed materials from the metabolic by-products, and these again from the secretory or excretory products. Absorption and secretion merge in each other. The higher we climb in the scale of organization, particularly in the animal kingdom, the more do we meet with cells which are entrusted with special functions. For instance, we find cells which receive matter from the exterior. Others transform particular compounds into products of a special nature. Others, again, have the function of carrying the final products of metabolism to definite points for excretion.

A unicellular organism stands constantly in relation with numerous substances of the outer world, which differ from place to place and from time to time. Some of these it makes use of as nutriment. Others, on the contrary, are entirely useless to the cell in question, while many would cause considerable harm, if allowed to penetrate its wall cells. To these substances the single cell does not yield itself helplessly, but has at its disposal various arrangements for its own defence. It has, in the first place, a cell wall which is impermeable by many substances. Further, the cell, by means of different processes, is capable of altering substances, which may in any way be injurious to it, in such a way that the active group is rendered harmless. Often a simple hydrolytic decomposition is sufficient to deprive the compli-

cated substance of its specific properties. The disharmonious product is decomposed into indifferent by-products which are harmless to the cell. More energetic means are often employed, and the material is oxidized or reduced *according to the special needs of the cell.* Even in these simple forms of life it is probable that many substances are rendered harmless by combining to form fresh compounds, just as, in the metabolism of a more complicated organism, rearrangements of different kinds are undergone which alter such materials as are undesirable, so that they may be excreted in this form out of the body. Very often a given substance is incapable of combination, in which case it must first be so transformed by special processes as to be susceptible to combination. We thus see how the cells of the body oxidize, reduce, or decompose, until a product is reached that is capable of combination. There is no reason to doubt that unicellular organisms have similar means of defence at their disposal, but they are not so easily traced, owing to the fact that it is more difficult to add certain substances to a single cell, without damaging it, than it is in the case of a more complicated organism. The latter are able to modify profoundly the action of substances introduced by the mouth, owing to the fact that they are gradually absorbed. Further, these substances are considerably diluted in the lymph

and the blood. Finally, they may be easily withdrawn from the body before they have had any opportunity of penetrating into the interior of the cells.

As a principal defence a single cell always has the cell wall, with its characteristic construction and its specific physical properties. Besides this, there is no doubt that ferments play a considerable rôle. They allow the cell to make a choice from amongst the substances which are continually acting upon it. These ferments, as Emil Fischer (Lit. 6)[1] has proved from his exact researches on the subject, are directed in a specific manner against definite substrates. Only those substances, which are capable of being decomposed by the cell into simpler groups, are in general found to be of use to it. Throughout, our positive knowledge has led us to the conclusion that cells supply their vital needs only from the simplest units of the nutritive material, and that they probably never break down such complicated substances as fats, polysaccharides, and proteins, directly into their final metabolic products. Even the simplest units are not at once completely broken up. The cell works in stages. First of all it splits up large molecules into smaller particles, and so sets free from the rest one fraction of the entire

[1] The numbers refer to the Bibliography given at the end of the book.

energic contents of the original material, until finally —at least, with carbohydrates and fats—the whole amount of contained energy is released. The cell regulates its own metabolism down to the minutest details. In the proper preparation of the material to be decomposed, and in the gradual liberation of the amount of energy required, lies the real importance of those substances formed by the cell, which we at present comprise under the name of ferments.

The ferments have yet another value for the cell, in that they help it to regulate its own structure. Not every product which is taken up by the cell passes into its structure. Sometimes the decomposition must be carried on further; in other cases the particles must be synthesized suitably for the production of the necessary structural unit; after which it sets about the recombination of all the numerous constructive units, so as to form the complicated characteristic structure of the cell. Though we do not yet know the precise nature of the ferments, yet their specific activities, and their great importance in regard to cell metabolism and cell structure, are well known.

Without energy no cell can do work or produce heat, and it is in energic metabolism that we find a true picture of the functions of the cell. How the cell procures the required energy, in what manner it makes use of it, and so on, we can learn only by

an accurate and possibly exhaustive study of the finer metabolic processes that go on in the cell. In these processes the so-called ferments play the most important rôle. With their aid we have succeeded in following up, outside the cell, processes which seemed to be exclusive properties of the cell. The more these experiments are extended, the more we meet with observations which show that we have been in the habit of picturing far too schematically the processes within the cell body. Thus, for instance, the simply formulated process of the fermentation of grape sugar into alcohol and carbonic acid gas— $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$—has been found to be a most complicated process. A whole chain of reactions takes place, before the grape sugar is finally converted into alcohol and carbonic acid gas. There are many more intermediate stages present than were ever suspected. It will be an important duty of future research to ascertain what importance alcoholic fermentation, in all its stages, has for the yeast cell. We are indebted to recent researches, in which Knoop, Neubauer, Friedmann, Embden, Dakin, Schittenhelm, Jones and others have taken a prominent part, for a knowledge of numerous intermediate stages in the decomposition of the amino-acids, of grape sugar, of purin bases, &c. Every time we demonstrate fresh intermediate links in the decomposition of certain compounds we get a

deeper insight into the working of the metabolic processes of the cells, and obtain important clues as to the means by which the cells of the animal organism produce, from compounds of a definite kind, substances that belong to a different group. We may mention here, for instance, the conversion of amino-acids into grape sugar, and of carbohydrates into fats.

Some of the unicellular forms of life, and some organisms consisting of a few groups of cells, are, at least in part, equipped with agents (ferments) which are not so precisely directed against certain substrates as the ferments of the higher species, such as plants and animals. While the ferments of the latter, so far as we know, principally decompose substrates consisting of units which are found in the cell-constituents that constantly recur in Nature, cases have been observed amongst the lower organisms (*i.e.*, morphologically lower) where the latter split up compounds, which have been prepared in the laboratory from units which are not known to exist in a free state in Nature. Owing to this wider independence these organisms are assured of better conditions of existence. These cells can live where others, being unable to secure the energic contents of the material supplied to them, and being also unable to form from this substrate the elements required for their bodies, are bound to perish through lack of nourishment. In

this way a cell dies, though it be plentifully surrounded by a material rich in energy, which, however, cannot be used because it lacks the proper form —both structural and configurative. It does not suit the organization of the cell. There is an abundance of oxygen at its disposal, but the latter cannot find any point of attack; and so the necessary preparation is wanting.

Some of the substances cannot be absorbed by the cell, simply because they are physically too coarse to penetrate through the cell wall. Such is the case with many colloidal substances, which must be first decomposed into simpler groups before they can pass into the interior of the cell. In these cases the presence of ferments is essential, and they have to be of such a kind as to be capable of decomposing the complicated molecules into a form which may easily penetrate through the cell wall. Often, however, such conditions may suffice as enable the coarse complex substance to be simply broken up into finer particles, which may be ingested in this form by the cell, without any molecular decomposition being necessary. Further decomposition takes place during absorption or, even later, at a suitable point within the cell.

Even a unicellular organism does not enter into intimate relations with substances which have not previously been remodelled. This remodelling

generally takes place in such a manner that the substrate is decomposed into simpler indifferent constituents, after which the cell builds from the base upwards.[1a] In many cases this rebuilding is unnecessary. Such is the case when the cell only requires the energy contained in the absorbed substance. As soon, however, as substances are required as vital units of the cell, then they have to be adapted to the whole structural plan in all its minutest details. This is also the case when secretory substances, having a characteristic structure and a specific action, are to be formed.

We know of unicellular organisms which produce their body substance from very simple elements indeed. Thus, we know of organisms which produce their cell plasma from carbonates, nitrates, water, and salts. Others can draw their nitrogenous supply from any substance which will supply them with ammonia. Others, again, make use of the free nitrogen of the air. There are, however, even amongst unicellular organisms, some that are very fastidious and will only thrive in the presence of certain peptones. Others even require certain forms of protein from which to obtain their derivatives.

An exhaustive study of the sources of nitrogen

[1a] See in connection with this, Emil Abderhalden, "Synthese der Zellbausteine in Pflanze und Tier," Julius Springer, Berlin, 1912.

necessary for each separate organism, paying due attention to other nutritious materials and conditions, will no doubt lead to exact methods for the culture of separate cells in the laboratory. Following this line of inquiry we could surround certain micro-organisms with peptones whose composition we are familiar with, and so acquire a deeper insight into the processes of their metabolism.[2] Even the mode of decomposition of the substrate, and of the intermediate stages, may furnish some important hints as to the specific functions of the cell and, in many cases, allow us to recognize particular organisms.[2a] We shall, by this means, understand why certain germs thrive upon certain media, while on any other substrate they either cease to grow or perish entirely.

It will also be possible to determine exactly, which of the decomposites and by-products formed from the culture medium produce harmful effects.

There is no doubt that, in the organic world, certain species prepare the soil for others, and in this way one organism acts as a pioneer to the others. It is a most interesting task to follow up this co-operation of different living beings in all its details. To a certain degree we have, in the co-operation of single

[2] If the nitrogenous basis from which different micro-organisms obtain their nourishment is not known, it might be possible to obtain a culture medium by decomposition of the micro-organisms themselves.

[2a] A firm at Höchst a/M. supplies peptones of definite composition for this purpose.

cells, a forecast of the division of labour found amongst the organs of the higher forms of life. In the former case we have the cells as yet free, while in the latter they are combined into tissues. From this point of view we may look upon the symbiosis of heterogeneous species of cells as a first experiment in the building up of a cell state. The single cells are still independent and their duties multifarious. There is no strong bond uniting the organisms into any one "organ," and yet they depend upon each other for mutual support. Unicellular beings begin to organize themselves into combinations. Another step further and we arrive at cell complexes having definite functions, which we call organs. But even the most developed organisms, both of the animal and plant worlds, have relations with cells which stand outside the common organization. By means of micro-organisms the plant gains access to otherwise inaccessible sources of nitrogen, while by means of bacteria the animals make use of the important carbohydrate, cellulose. The bacteria convert the latter, within the intestines, into products which can be further decomposed by the ferments secreted by the glands.

In those organisms in which division of labour has been instituted amongst the cells, and particularly in those in which definite cells have closed in to form an alimentary canal, these are the only cells which

are in communication with the outer world. They alone know, so to speak, what food is ingested. Even these have no direct relation with the ingested material, seeing that the latter, before being taken up by the cells of the gut, has been subjected to the action of the ferments poured into the alimentary canal, and been disintegrated into simpler and indifferent particles. All nutriment of a composite nature is dissolved in stages, until finally products of decomposition result which no longer exhibit any special characters.

Generally speaking, food supplies the material for the building up of the cell, and we must remember that we are dealing with the complicated tissues of animals and plants. Each cell has a specific fabric of its own, which is dependent on the nature of its separate units, and on the manner in which they combine together. We must not look upon this from a purely chemical point of view alone, but should pay attention to its physical aspects as well. The sum of the properties resulting from the special structure of the cell conditions its special functions. When such cells, with their specific structure and functions, are taken in by an individual organism, the latter can at first do nothing with the material supplied. The special character of the different products that make up the particular cells must first be destroyed. Unit must be separated from unit, so as to leave only a mixture of simple

compounds, from the elements of which the body cells may construct their own material, or else renew their supply of energy. In the latter case, too, as has been mentioned before, a preparatory decomposition (a kind of adaptation to the cell) is necessary.

An analogy may be used to make clear this kind of reconstruction. Suppose an architect is called upon to convert a certain building, which has been specially designed for a particular purpose, into one suitable for an entirely different object. He would only be able to carry out this work on the condition that he might pull down the original structure. He would naturally be able to work some of the bricks of the old building into the plans of his new one. Some of the bricks, or even combinations of bricks, may be used as they are, others will have to be recut, while others, again, are of no value whatever. In just the same way does the animal organism act towards the specifically constructed parts of the cells which are used as food. First of all comes the disintegration into simple compounds, and then a reconstruction, according to entirely new plans, on the other side of the intestines.

The simplest conditions, in this respect, are to be seen amongst mammals during the suckling period, when, under normal conditions, the animal imbibes the milk peculiar to its species. This, as G. von Bunge first demonstrated very precisely, is in every

respect adapted to the growing organism (Lit. 2, 3). The great point is, that the suckling is constantly supplied with the same mixture of salts and the same organic nourishment, namely, albumen, carbohydrates, and fats. Later, when mixed nourishment is taken, the conditions become much more complicated, according as greater quantities of this or that unit are introduced during digestion. The cells of the intestines are continually confronted with new duties, and have to adapt themselves gradually to the new conditions.

The cells of the milk glands are charged with the proper choice of food. They prepare the food for the developing organism, and simplify in particular the task of the intestines which, with the help of the liver, prepare the ingested food for the other cells of the body. Even the components of the milk, before they can be of any use to the organism, must be first considerably altered in the intestine, just as later, in the case of mixed food, a complete decomposition by means of the ferments precedes absorption. The difference in respect to the latter mode of nourishment only lies in the fact that, with the milk food, the same stages of decomposition, giving rise to the same by-products, always recur. Day after day, to a certain extent, the cells of the intestines and of the organism have to perform the same task.

From this point of view we may discern three

important stages in the nutrition of the young of the mammal. Right up to its birth, which is the first stage, the fœtus has received from the mother only food which is in harmony with her body, and it makes this harmonize with its own blood and its own cells. Its organism has never come into contact with entirely disharmonious substances, and thus its metabolic processes run on definitely balanced lines. But birth supervenes, and with it the first change in the mode of nourishment. The individual has become independent. Respiration begins, and the cells of the lungs immediately enter upon their duty of exchanging gases. With equal rapidity the cells and glands of the intestinal walls undertake their new functions, which are, with the help of ferments, to prepare new nourishment for the cells of the body. The mother facilitates this task by giving off a supply of milk that is adapted to the requirements of the infant. In the first place, the intestinal cells have their task simplified. They never come into contact with a continually changing mixture of ions, nor are they overwhelmed with all kinds of disintegrated organic by-products. In this way the as yet inexperienced being is gradually accustomed to its new functions, and finds itself at last well prepared when it has to deal with a new kind of food which requires it to exercise its functions in a more variable, and, consequently more difficult manner. From the moment of parting

with the milk as sole nourishment, from the moment of passing on to the mixed nourishment that is peculiar to its species, the second important change in the feeding of the growing individual is accomplished. The third stage of its evolution has begun.[3]

The cells must function quickly to prevent disharmonious substances from entering the circulation. To ensure the proper discharge of a duty so important to the organism, the liver is placed between the intestines and other organs. Within this important organ the blood, still laden with the absorbed and partly metamorphosed food-stuffs, comes into contact with the liver cells. This material is once more thoroughly sifted, and the blood is finally discharged into the general circulation, freed from all substances that would be out of harmony with the body and the blood.

The knowledge that digestion is the means by which unsuitable products are prevented from passing into the blood and the cells of the body is of the greatest importance for our comprehension of the whole metabolism of the animal organism. Thus, to a certain extent, we may look upon the animal organism as a whole in itself. All the cells of the

[3] From this point of view it is easy to see why lack of its proper milk sets up disturbances in the suckling, and particularly how dangerous are continual changes in the composition of the food, seeing that the young animal is not yet prepared for the reception of mixed nutriment.

body have a common architecture, which is bequeathed from generation to generation by means of the sexual cells. The cells which combine into an organ have, besides that, a structure specific for the organ. We are bound to accept this view, otherwise it would be incomprehensible why, for instance, the cells of the liver should produce only bile, and the cells of the medulla of the suprarenal bodies adrenalin, &c. All the cells of the body have certain functions to perform which are of use to the whole organism. It is quite certain that the different organs supply substances to the blood, which set up definite processes in other particular parts of the organism. If these substances are to act effectively, they must have a definite specific structure. The cells, too, on which they are destined to act, must also be characterized by a special structure, otherwise it would be difficult to understand why a special secretion acts only upon certain cells, and leaves a number of other cells quite unaffected.

A particularly fine example of the specific action of gland secretions upon cells of specific structure is supplied by such cases of hermaphroditismus verus as that, for instance, in which the bullfinch is found to have a testicle on one side and an ovary on the other. These peculiar animals have on the one side male, and on the other female, plumage, each being delimited accurately, and without any transition, along the middle line of the body. It is absolutely

impossible to imagine that the gland secretions of the two different glands, which bring about the full development of secondary sexual characters which are obviously present from the first, should remain only on one side of the body. They must, in fact, be carried by the blood to all the cells of the body. Nevertheless, the secretions of the male gland pass only to those cells which have "male" properties, and vice versa, the secretions of the ovary affect only the cells of the "female" half of the body.

Strong support for this view of a specific cell structure is supplied by the numerous experiments on transplantation. The surgeon nowadays tries, as much as possible, to retain the full strength of the functions of every organ, and, if some of the tissues are missing, he seeks aid in substitutes. It is found that only those tissues graft which are taken from the same species, while still better results are obtained by the use of parts of the same individual. Heteroplasty, *i.e.*, the attempt to graft foreign tissues, has never succeeded. A body requires cells in harmony with itself. If they are in close relation, as is the case in tissues of the same species—even the individual has its own type—then it is very probable that with time the newly grafted tissue will, by means of reconstruction, assimilate itself with the other cells of the same organ, and so eventually with the entire organism.

Finally, pathology provides us with a large number of cases supporting our view of the specific structure of the different cells belonging to a given organism. We know that certain poisons have an injurious effect only upon very definite kinds of cells. We might here refer to the well-known system diseases of the central nervous system. The so-called metasyphilitic phenomena, for instance, manifest themselves, only in very special regions of the spinal cord and the brain.

The idea that each kind of cell has its own structure, and to some extent its own metabolism, opens up a wide vista for therapy also. So far as the organism always forms products which act upon certain cells and only upon these, it must be possible to find substances which will act only upon those cells whose metabolism we may wish to alter in some way or other, or whose complete destruction is desirable. The latter is the aim of the battle waged against germs of infectious diseases and tumour cells, especially cancer. There is a great future for cell-specific therapy, which will pay special attention to the structure and the configuration of the means employed, or else attempt generally so to modify the chemical and physical conditions in certain cells that life will be impossible for them.

The admission of a certain specific structure, for each cell species with special functions, implies that each separate cell possesses special means enabling it

to regulate its own structure. The components of the blood plasma, which serve as the deriving material, are the same for all cells. The formation of a specifically acting secretion also requires that every kind of cell should have means and arrangements at its disposal for the specific transformation, under certain circumstances, of the same product. From this point of view we should expect to find that each kind of cell controls particular ferments, of which, however, some will be common to all the cells of the body. These ferments have the task of decomposing the nourishment, brought by the blood plasma to the cell, into simpler products. Investigations on the peculiarities of cell ferments—the tools of the cells—are already in progress, and we shall deal with this question later on. It may be that the result of these investigations will supply the most unequivocal and sure support for the theory of the dependence of cellular function on cellular structure.

For the maintenance of a regular and undisturbed flow in the varied processes of the cell, we must assume that within certain limits constant conditions prevail. When we carry out certain experiments in a laboratory and try to study, for instance, the interaction of two substances upon each other, we choose the most favourable conditions possible, and take particular precautions against the presence of any

other substances than those essential to the reaction. It is a well-known fact that the slightest contamination may influence the reaction to a very great extent. It may either fail altogether, or be retarded, or may even be diverted into quite a different direction. We meet with great difficulties if we have to follow up several reactions in one and the same medium. Intermediate products may act, one upon the other, to such an extent that we arrive at a series of final products whose origin it would be extremely difficult to account for. Now, if in an animal organism the separate processes were not regulated in a very strict manner, and if, for instance, the blood did not receive substances which are in harmony with it, that is, always transformed in a definite and regular manner, it would be difficult for us to understand how the separate secretions always attain their aims in a very certain way, and how they are able locally to attack particular metabolisms, and either retard, or hasten, or initiate them.

There is not the slightest doubt that the course of this metabolism, as well as the inter-relations of the cells of a particular organ, is only imaginable under the supposition that the metabolism of the whole organism is regulated in the most precise way, not only quantitatively, but also qualitatively. We are bound to imagine that, in the work of the cells, the same stages of decomposition recur regularly, and

that it is at a quite definite stage that the by-products of metabolism are passed by the cells into the lymph channels, and so into the blood system. The individual cell is in this sense responsible for the constant composition of the contents of the blood, in the same way as the cells of the bowels with their respective ferments.

Here, again, the animal organism controls important weapons of defence which may correct any possible errors. Between the blood and the cells of the body lies the lymph. The latter is the first to receive the substances supplied by the individual cells, and controls them by means of its accessory apparatus, namely, the lymph cells and the glands. Some of the substances are further disintegrated or transformed in some other way, and, perhaps, even utilized for various syntheses. From this point of view we may look upon the lymph as a powerful means of defence, whose aid is particularly valuable in preventing the infusion into the blood of compounds that are both quantitatively and qualitatively unsuitable. From all sides care is taken that only normally suitable substances shall appear in the blood.

From this point of view we may distinguish substances that are "out of harmony with the body," *i.e.*, such compounds as, in their structure and configuration, show no correspondence with the con-

stituent parts of the organism. To these belong all such substances as are received from the outside as nutriment, with the exception of those products which may be ranged amongst the most simple units, as, for instance, grape sugar. As substances "in harmony with the body," we would then term those which, when entirely recast, correspond in their structure to the essential composition of the particular species or individual. In addition to this general conception, which only means that a substance is not absolutely disharmonious to the body in general, we have undoubtedly to make a still finer distinction according to the special features of the compound in question. As early as the year 1906[4] we had suggested the advisability of distinguishing between substances which, though they are adapted to the blood, are nevertheless out of harmony with the varied cells of the body, and those which show any features characteristic of the structure of the cells of a particular organ. If our ideas concerning the structure of the particular cells of an organ, and the dependence of its functions on this peculiarity, prove correct, then it follows that, as we have already emphasized, each kind of cell must have at its disposal units of its own kind. We may then speak of substances that are "in harmony with" an organ,

[4] "Lehrbuch der physiologischen Chemie," 1 Auflage, S. 292, Urban and Schwarzenberg. Berlin-Wien, 1906.

or even more precisely, "with the cells"; or "with the blood." Substances that are specifically elaborated for the blood would then be "out of harmony with" the cells, and conversely the substances "in harmony with" the cells are "out of harmony with" the blood, or better, with the plasma, because the components of the form elements of the blood are out of harmony with the plasma, and inversely. Products in harmony with the cells will only be in harmony with one another in so far as they belong to cells with similar functions, so that from this point of view, for instance, the specific elements of the thyroid gland must be regarded as out of harmony with those of the suprarenal bodies, and inversely. The idea of an entirely specific structure for each cell of an organ—both from the chemical and physical points of view—is based not only on the supposition that, without such a notion, the special duties and functions of the separate cells of the body would appear incomprehensible, but, above all, on the above-mentioned fact that definite secretions given off by particular organs act constantly and only upon cells of a definite system. This implies that the cells in question must have a structure which distinguishes them sharply from all other kinds of cells.

The view that each animal species is capable of building up complicated compounds of peculiar

structure, and further, that every cell with special functions is formed of specially constructed components, is very often met with doubt. How is it possible for the animal and plant worlds to produce such an enormous number of different compounds? There would have to be formed millions and millions of different substances. Only think of the enormous amount of animal and plant species, and just put against this the fact that in general always the same and similar components reappear! In each cell we meet with carbohydrates, fatty substances, and albuminous particles. If these compounds are decomposed into their units, we find the same compounds resulting. All the albumens give, for instance, with very few exceptions, the same, that is, some twenty amino-acids. This obvious contradiction—on one side cell constituents based on similar elements, and on the other the idea of specifically constructed cells—disappears immediately we begin to make a calculation. Suppose we synthesize three elements A, B, and C; we at once obtain, by merely altering the sequence of the particular combinations, the following six different products:—

A—B—C	B—A—C	C—A—B
A—C—B	B—C—A	C—B—A

If we start with four different elements we get twenty-four different compounds, while five elements

correspond to one hundred and twenty isomeric combinations. We give below the number of possible compounds which result from simply altering the sequence, the form of combination remaining the same.

The number of different units	Number of resulting compounds, the sequence only being changed
8	40,320
10	3,628,800
12	479,001,600
15	1,307,674,368,000
18	6,402,373,705,728,000
20	2,432,902,008,176,640,000

This enormous number of different compounds is solely produced by the manner in which the twenty elements follow one another. If hydrolysed, all these compounds would give the same elements to the same amount. These reflections may serve as a warning to those investigators who are inclined to infer the identity of particular compounds from the presence of the same elements.

Nor is it only the sequence of the individual units that needs to differ; for the mode of combination of the different compounds may also vary. The number of possible combinations is infinite. Again, the units are present in unequal quantities. Finally, one very important factor must be allowed for. No cell is composed of only one albumen particle, one carbohydrate, and one fatty substance; on the

contrary, we always find mixtures of these. So that, given quite similar compounds, *e.g.*, several albumens, the cell has the power of making up mixtures of various kinds which give it a special stamp. By these means we see then that the possibilities for the production of specifically constructed kinds of cells are infinite. No one would be able to calculate the number that would account for all these possibilities.

We take it as probable, on the strength of numerous observations, that all through the animal kingdom similar organs show, besides their specific, and possibly individual characters, certain features which are common to all species of animals. All that is required is the recurrence of a particular albumen in the cell. We conjecture this from the fact that experiments have shown that certain ferments, when they act on albumen of a special kind, show specificity for the organ, but yet are not specific for any particular animal species. It is probable that we are here on the track of an important biological law.

Yet, in spite of these similar or kindred features, each species and individual retains, by means of the mixing of its cell components, the cell organization peculiar to its kind. If a single group be repeated but once only, the ferment that acts on it finds a point of attack. We lay stress on these points, because a casual consideration of the fact that in the

dialysation process, as well as in the optical method, human organs may sometimes be replaced by those of animals, might easily lead one to argue against the existence of specifically constructed cell units, as well as of the ferments that act on them.

A special place is occupied, at least qualitatively, by all those substances which, like the units of the different organic nutritive and tissue materials—as well as the inorganic constituents, the salts, water, &c.—exhibit no specific structure, and which are common to the most different kinds of cells, as well as to the blood and lymph, as intermediate and final products. In this case disturbances can only be caused by quantities. Rapid secretion, or synthetic or analytic processes, may in such cases act in a regulating manner and again restore normal conditions. All substances, however, which have a specific structure, are peculiar either to the blood or else to specific cells. From this point of view we must consider substances, which leave the cell and pass into the blood in a state of insufficient decomposition, as being out of harmony with the blood, or rather with the plasma; and, inversely, disturbances would certainly occur in the metabolism of certain cells, if, for instance, the insufficiently decomposed constituents of muscle cells were to penetrate the cells of the kidneys. The units of the muscle cells are out of harmony with the cells of the kidneys, and only a

radical reconstruction could make them harmonious therewith.

That, in an animal organism, the formation of material for definite cells can be effected by the components of absolutely different cells, we can learn from experiments on the starvation of animals, and particularly from the well-known observations made by the Basle physiologist, Friedrich Miescher, on salmon. This observer was able to prove that the sexual glands of this fish become extraordinarily developed in fresh water at the expense of the muscles. It can be demonstrated microscopically that the components of the muscle tissues are gradually decomposed until they pass into the blood circulation; and Miescher speaks quite plainly of a liquidation of the units of the muscle cells. At the same time it may be observed that the sexual glands gradually begin to grow, without the animal taking any nourishment. But in the cells of the sexual glands we do not meet with the specific muscular constituents in an unmodified state; on the contrary, we meet with quite new substances, chiefly albumens in a state in which they are never met with in the muscle cells. We notice in this case that histones appear in place of the muscle albumens. These are albuminous bodies of a basic nature, containing the so-called di-amino-acids in large quantities. Soon we find the histones, the more the sexual organs, and

especially the testes, approach maturity, replaced by protamines, which consist nearly exclusively of diamino-acids. We see, in this example, how cells of a characteristic structure transfer their material to the blood circulation in a profoundly modified form. First of all substances are produced that are in harmony with the plasma, and these are transferred to the cells of the sexual glands by means of the circulation. These glands take up the indifferent substances, and from them build up products specific to themselves. There is no doubt that similar processes play a part in normal metabolism. Sometimes one group of cells will help another in this way, particularly in cases where the supply of nourishment is delayed for some time.

The reconstruction of substances of every kind from products that are harmonious to the plasma and the lymph is demonstrated by every growing hair and every growing nail. Every new blood corpuscle tells us of far-reaching transformations; and every secretion—whether produced directly, as in the case of saliva or milk, or manifested when a fistula is produced by surgical means, or whether it forms a so-called internal secretion, choosing the blood or the lymph for its path of action—every one of these gives evidence of powerful disintegrations, integrations, or transformations. When thousands and thousands of leucocytes hurry forth against an invasion of

micro-organisms, for the purpose of limiting their sphere of action or of subduing them, no more convincing picture could be presented to us of the synthesizing capacities of the animal organism. Even the full-grown organism is able at any moment to completely equip a vast army of cells and endow them with special functions.

If the ingested food materials, with their peculiarly disharmonious structure, were passed directly into the circulation and handed over to the cells in this state, then the organism would be subjected to continual surprises. The control of its metabolism would be utterly impossible under such conditions. Sometimes one substance, sometimes another, would predominate in the circulation, and the blood would be correspondingly affected sometimes in one way, sometimes in another. The cells would have to disintegrate all these disharmonious materials. In such a case they would have to be provided with all sorts of arrangements for the continual modification of these materials. Each separate cell of an organism would be in exactly the same state as a unicellular organism. Just as these have to make a selection from amongst the disharmonious substances by which they are continually bathed, so, too, would the cells of the body have to pick out the substances they need, according to the conditions presented. Not only would the work of the single cells be enormously

increased, but also, without doubt, the mutual influence of different kinds of cells, by means of certain secretions, would be much hindered. And not infrequently we should find that some substance, that was quite specific in its structure, would be caught up by disharmonious substances circulating in the blood, and would be either altered or completely annihilated. In a short time the extraordinarily delicate regulation of the general metabolism would be thrown out of gear, and all kinds of injuries would inevitably result. The intermediate products in particular, which may vary in any given case, would give rise to disturbances.

The cell, as has already been mentioned, always works by degrees, for it is quite incapable of suddenly decomposing a complicated molecule, and of directly transforming it by means of combustion into its final products. The cell builds step by step, and so preserves the equilibrium of its energic metabolism. The rapid combustion of albumen, fats, and polysaccharides would, in certain places, suddenly produce a great deal of energy, which would appear in the form of heat, and under certain circumstances would destroy the life of the cell itself. In consequence, the gradual acquisition of the energic contents of the food is of the greatest value for the maintenance of all the finely graded processes of metabolism, as well as for the functions of the individual cell; while, on

the other hand, the decomposition of some disharmonious, unsuitable, material may give rise to some intermediate stages which are the cause of serious disturbances. Here and there a cell would be seriously injured. Complete disintegration could never be effected, either because the cell would refuse to act, or because it would lack the particular agent with which to dissociate the compounds presented to it. All this would lead to numerous possibilities, which would exclude all regularity in the metabolism of the cells, as well as in the general metabolism of the body.

The animal organism prevents all these possibilities by allowing only material which has been put in harmony with the body, and particularly the plasma, to reach the circulation. The nutritive material of the tissue cells, which from this point of view can be considered homogeneous, gives decomposition stages with which the cells have been long familiar. Nothing that is disharmonious appears on the scene. Just as in a workshop, in the production of an article, one machine prepares the material for another, and one workman transfers to another material which is finished up to a certain degree, so do the tissue cells mutually support each other in their task. The cells of the gut and the liver continually act as important sorters for the whole organism. One may imagine the chaos and disturbance which would be

produced in a workshop if machines were suddenly supplied with unsuitable material. All of them would soon refuse to work and come to a standstill. The single workman, who, with his knowledge and his tools, is trained only for a single phase in the production of a complicated whole, would be helpless if he were suddenly ordered to undertake a new task. He would require new tools, and be forced to acquire new experience. If his duties changed without any regularity at all, *i.e.*, were he restricted in his activities to any casual work that might be given to him, then any successful results would be entirely out of the question. We find exactly the same relation in the collective mass of cells which compose our organism. The single cells represent the machines and the workmen who, in an enormous workshop, pursue common aims in separate groups. The cells of the gut and its accessory glands, especially those of the liver, superintend in a certain degree the supply of raw material, which is first prepared in a proper manner, and then recast so as to be "palatable" to all the cells; after which it passes from hand to hand—from one cell to another.

In these considerations it is not only the purely chemical processes that have to be taken into account; the physical processes also play an important rôle. Every cell possesses substances which have an

influence upon osmotic pressure, together with others which are without this influence. In this respect, too, the cell is always laid down on the most delicate lines. Sometimes it decomposes colloidal substances and transforms them into others, which increase the osmotic pressure of the cell; at other times it synthesizes materials in solution into larger, more complicated molecules, until a body appears which is more and more extracted from the solution, and by this means loses its influence upon the osmotic pressure of the cell. This variety of function is of great importance to the cell in quite a different direction. We know that single ions exhibit very specific activities. Here also the cell must be equipped with arrangements to accelerate in one case the action of a separate ion and to check those of another, or else to entirely exclude them. The cell is able to effect this in diverse ways. Sometimes an ion is combined with a protein, for instance, or with other substances, and so is robbed of its own characteristics; at other times an ion is set free through decomposition or simple dissociation. Or else the cell induces antagonistically acting ions to react mutually on each other in finely graduated stages.

Numerous experiments have shown, as has already been mentioned, that definite cells depend upon definite secretions having their origin in other organs. If we remove certain organs, for instance the thyroid

gland, the accessory thyroids, the sexual glands, the suprarenal bodies, and so on, we get definite degenerative phenomena appearing. In many instances, indeed, the absence of these organs is incompatible with life itself. The same phenomenon manifests itself when the organ is left in its proper place, but through some cause or other gradually discontinues its proper functions. In such cases there is no need for the organ to be destroyed; it is sufficient if the production of a specific secretion entirely ceases, a condition which is equivalent, to a certain extent, to the complete absence of the organ. These observations, which are supplied to us by pathology, together with facts which may be produced at any time—as when we extirpate certain organs and, after the results of such extirpation have manifested themselves, make a fresh transplantation—give an extremely varied picture of the reciprocal relations of the different organs towards each other.

Each group of cells—each organ—has certain functions to fulfil in regard to the rest of the cell organization, and in this respect it possesses a certain independence of its own. There are also, of course, reciprocal relations within the cells themselves of an organ. Many observations point to the possibility that apparent morphological unity of an organ does not always mean unity of function. The independence of a given organ is only a relative one. As we have

repeatedly indicated before, all the cells stand in actively reciprocal relations with each other. We have plenty of proofs for the acceptance of this view; while, on the other hand, we have no clear insight, at present, into the signification of this reciprocal dependence. Probably unicellular organisms alone are wholly dependent upon themselves. They perform all the processes necessary for life independently of other cells, except when, as sometimes happens, a conjunction of these simple organisms rises to the level of a symbiosis. The latter, as we have already pointed out, must have a value corresponding exactly to the reciprocal interactions of the cells of the more highly organized forms of the vegetable and animal kingdoms. For there is no doubt that in plants, too, the cells have actively reciprocal relations.

Doubtless there are, in an organism composed of cell groups, numerous kinds of cells which can live without having reciprocal relations with other cells, exactly in the same way as a single individual can isolate itself from its stock and still continue life for a certain time. But in the same manner as the well-being of a people or a State finally depends upon the regular collaboration of the many, so each kind of cell expresses its full value only by associating its work with that of the other cells in the organism. Only then is a cell capable of developing all its capacities. In many particular functions, indeed,

so much division of labour is found, and to such an extent, that a large number of cells are entirely dependent upon the functions of others. Were such cells to cease to work this would result, as has already been mentioned, in the sickness and finally in the death of many other cells. In this direction there still lies an extensive field of research before us. The "whys" and the "wherefores" in this case extend indefinitely.

The possibility of breeding single cells and pieces of tissues in the blood plasma outside the organism, and keep them alive for a certain time, opens out a prospect of answering many problems by experimental means. We shall see in due course why some of the cells lose their normal functions when the secretion of certain organs is lacking. The number of possibilities is almost unlimited. For example, some substances, such as grape sugar, can only be dissociated by the cells into final products—carbon dioxide and water—after they have been prepared in a certain manner. A gradual dissociation takes place. The cell is equipped with appliances for the alteration of a given substance, but they are not at first in a condition suitable for use. A second agent must first of all make them capable of their respective functions—just as a hammer without a handle, or a screw without a screwdriver, are only useful when the missing parts are at our disposal.

These agents are probably supplied by the cells of other organs.

It is quite probable that, at present, being too much concerned with the phenomena of structural chemistry, we observe the processes in the cell from a too one-sided point of view, and think too little of the physical state of the cell. We know that many reactions depend entirely upon the conditions present, if the action is to take place. For instance, a change in the reaction of the medium is sufficient to annihilate the activity of a ferment. The addition of the least trace of an electrolyte will, under certain circumstances, accelerate certain reactions; and alterations in the conditions may even upset a reaction entirely, and lead to totally different end products. The processes in the interior of the cells are surely subjected to a much greater extent to the influences of the physical state of the cell. Colloidal substances and electrolytes—the ions—and perhaps the rest of the substances in solution, certainly play a considerable rôle in their reciprocal relations. Here we meet with regulations of a kind which we are at present unable to discern. Might it not be in this direction that the collaborations of different body cells would appear of the greatest significance? Many a process, which manifests itself and attracts our attention most strongly on account of the ease with which it can be demonstrated, may perhaps be of quite a secondary

nature. The cause—the primary process—escapes our notice, partly because at the time we do not know how best to state the problem, partly because we have no methods at our disposal for an experimental investigation of the case.

In all biological problems it is remarkable how entirely dependent we are upon the philosophy and the methods employed in the exact natural sciences. We transfer all that is there obtainable to the problems of biology. For some years certain ideas prevail, only to recede as soon as a new impulse or a new success in the domain of physics and chemistry directs a host of workers into new paths. We drill and work until a new gallery is driven into the rock of puzzles which is found in every cell. Very often the gallery ends blindly, but on its way has given rise to numerous interesting discoveries. Sometimes, however, the pioneer work is crowned with success. An important stage is left behind, and a new outlook gained. The final aim, however—a complete insight into the metabolism of the cell—still lies far ahead. Yet the knowledge we have acquired serves as a compass to keep us on the right road. The careful traveller will never leave anything unnoticed, for observations which often seem but trifles may point the way to entirely new problems.

In studying the functions of the cell we must never forget that there is not a single substance which is of

no value to the cell. It would be quite erroneous if we were to consider any substance—for instance, albumen—as the paramount life substance. A single ion can in certain cases decide the life or death of a cell. An aggregation of molecules may combine to form a powerful complex—a colloid—and by means of its properties dominate the whole function of a cell. The structure and configuration of the separate compounds, and of the separate units of the cell are of the greatest importance for its individuality. To this we must add, and as partly conditioned by the above, their structure and configuration in the physical sense. A separation of the chemical and physical properties of the cellular units is impossible, since they constitute mutually the conditions of life for the cell. They stamp it with its own character.

Substances, which may be indifferent products for one kind of cell, may be injurious to another kind. Each cell produces secretions of its own, in the formation of which many intermediate stages are passed through. If the whole transformation into substances that will be in harmony with the plasma be performed inside the cell, then any by-products that may appear, even though they be not indifferent in regard to other cells, will display no injurious activity in the organism as a whole. If, however, such insufficiently transformed substances penetrate into the general

circulation, then we must expect troubles of all kinds. Such a case may arise, for instance, when certain cells cannot complete a decomposition that they have initiated, owing to the absence of the necessary agent, *i.e.*, the ferment; so that the incomplete action of a particular organ may be the cause of numerous disturbances of every kind. If continuity of function be broken but once, then one disturbance, like an avalanche, is followed by another. It is true that the organism defends itself in such a case. It produces compensatory activities and tries to adapt itself to the new conditions, often succeeding in a most amazing fashion, and repairing the damage for a long period of time. Pathology supplies us every day with examples of this kind. The study of cellular functions under variable conditions is one of the most attractive that we know. Experimental pathology is a field which will be of undoubted importance for the whole of physiology, and to an extent as yet unrealized.

Thus all observations on the structure and metabolism of the individual cells of the body lead us, in the most unequivocal manner, to the conclusion that within a given organism large aggregates of cells work together harmoniously for the benefit of the whole. Complete harmony of relations is guaranteed—let us emphasize the fact once more—by

having on the one hand the cells of the gut and the liver ready to prevent anything, that is not completely deprived of its own characteristics, from passing into the circulation, and, on the other hand, by having the cells of the body passing on to the blood only such substances as have been so far disintegrated as to have lost those features which harmonize them with the cells. Blood which is in circulation thus always shows the same metabolic products and the same substances; and from this point of view we may consider the contents of the blood as being always constant. No doubt the duty of the lymph, which is placed between the cells of the body and the blood, is to guard the blood against an excess of individual products of metabolism. Probably, also, some of the products, which have been insufficiently disintegrated, are finally decomposed by the lymphatic glands, or by the lymph itself.

We are bound in this sense to look upon the lymph system, as indeed we have already pointed out, as an important control station. By means of its own cells, and especially by means of the glands, the lymph watches that no material shall reach the blood which is out of harmony with it.

From the above point of view we gather an insight into the significance of the invasion of organisms of all kinds into an animal organism. The isolation of the whole organism is immediately disturbed when

disharmonious cells settle on any spot within the hitherto harmonious cell complex. From this moment the harmoniously organized cells of the tissues are subjected to the influence of a kind of cell which has an utterly strange organization of its own. These new cells have a characteristic metabolism corresponding with their whole structure and configuration, and this they bring with them definitely into the new organism. They pass into the blood numerous end-products of their metabolism. Further, some cells decay here and there, and partial products reach the blood which are out of harmony with the species, and, of course, entirely so with the plasma and the cell. The whole regulation of the normal metabolism is seriously injured. The cells of the gut-wall will still be on the watch to prevent any disharmonious material from entering the organism; and the single cells of the body will still struggle to supply the blood only with properly altered substances. But the whole organization has been damaged, in regard to the collaboration of its various cells, by the fact that disharmonious substances are continually given off by the invaders. The very same thing occurs if, through any cause whatsoever, the cells of the body change their structure and acquire a metabolism which is entirely foreign to the rest of the cells of the body. If cancer cells or sarcoma cells, for instance, appear, then we

have cells before us which are neither subordinate to, nor co-ordinate with, the rest of the complex of cells. These cells have obviously reached a definite state of independence, nor do they maintain any direct relations with the different cells of the body. They are, so to say, outside the association of the cells of a particular organ, nor is there any doubt that they produce secretions, the products of their metabolism, which are out of harmony with the blood plasma. And we can well believe that here, too, cells decay, and products pass into the blood which are quite out of harmony with the plasma.

These ideas afford the possibility of studying, within the body, the action of disharmonious organisms of every description, especially of micro-organisms, and their relations towards the rest of the body cells, from a purely physiological point of view. It seems to us well worth while to follow up these conceptions, and to attempt, by means of direct experiments and observations, to bind together in closer relations the two fields of research that are covered by physiology and the study of immunity.

In the first place, we set ourselves the question: To what measures does an animal organism resort if substances penetrate into its body, and particularly into its blood, which are out of harmony with the species as a whole, or else only with the blood or

plasma? Is it deprived of the possibility of defending itself against such substances, or have the cells of the body also, excluding those of the intestines, retained the capacity of attacking complicated substances which are out of harmony with the organism, and of reducing them by profound decomposition to indifferent particles, which the cells may use for the construction of new material, or else as a source of energy?

To solve this problem, in a satisfactory manner, preliminary experiments on a very large scale were required. First of all, it was necessary to ascertain in what manner the individual cells of the body use up the nourishment which is normally brought to them by the blood. Does the individual cell decompose the complicated nutritive material directly into its end-products, or does it always disintegrate them first into simpler fragments, which are then reduced by successive stages, until finally the whole of the stored energy which the organism is capable of setting free is at the disposal of the cell, and the final products of the decomposition appear? All experiments that have hitherto been carried out in this direction lead us, as we pointed out at the beginning, to the idea that each separate cell of the body in general, with very few exceptions, disposes of the same, or of similar, ferments as those secreted by the digestive glands into the intestinal

canal. These ferments may not be identical in all details. It is quite possible that the ferments passing from the glands of the intestinal canal differ more or less in nature, because, in the case of food, a much more heterogeneous mixture of separate products is introduced from the outside than is found in the already transformed nutritive material of the cells of the body, which circulates in the blood and lymph channels. It is also possible that differences prevail in the mode of disintegration, and consequently in the resulting decomposites. It is quite certain that the cells of the body are capable of hydrolytically splitting fats into alcohol and fatty acids. Further, they are able to decompose carbohydrates of a complicated structure, especially glycogen, through dextrines to maltoses. The maltose formed is reduced, by the ferment known as maltase, into two molecules of grape sugar. We know also that very dissimilar cells of the body contain ferments which decompose albumen into peptones. The latter are further reduced to still simpler products, and eventually amino-acids are left, which again may be subjected to further reductions.

It could, further, be easily shown that the cells of the body are able to decompose into their structural units the so-called polypeptides, that is, amino-acids linked in the manner of acid amides. These ferments have acquired the name of peptolytic ferments. Their

presence has been demonstrated in animals and plants inside the most varied kinds of cells. In plants they are not always found in an active state. In seeds, for instance, they appear only when these are beginning to germinate. In the same way they are absent, as Iwanow has shown at my Institute, when plants are resting during the winter. In the fœtus their presence can be demonstrated fairly early. They can be detected, for instance, in a chicken on the seventh day of development, while in embryos of swine active peptolytic ferments appear on about the fortieth day.

The demonstration of the peptolytic ferments may be performed in various ways. One way is to treat them in the manner adopted by Edward Buchner, namely, to entirely destroy the cells of certain tissues or even single cells by trituration with quartz sand, so as to squeeze out the internal fluid of the cells. This fluid is afterwards mixed with kieselguhr, which readily absorbs moisture from the cell fragments, and produces a compressible plastic mass. The absorbed juice is then extracted out of the latter under pressure—up to 300 atmospheres—and filtered through a filter candle. We get a clear juice, which contains many components of the cells; the original structure of these having, of course, disappeared. In a juice obtained in this manner the presence of various ferment activities can be demonstrated, and it may be

shown that many processes go on exactly in the same way, qualitatively, as if the cell were intact. But the principal life process, the oxidation to carbon dioxide and water, is not found. Even slight injuries to the cells are sufficient to annul this important process. In such a juice it may be said that only the preparative functions remain—all of them processes which we usually ascribe to ferments. If to the juice obtained in this manner a peptone containing very sparingly soluble amino-acids is added—as, for instance, tyrosin or cystin—or else a kind of peptone in the building up of which an amino-acid takes part—and this may be easily detected at the moment of decomposition by means of a colour reaction[5]—then it is very easy to ascertain whether the juice contains any ferment that is capable of splitting the peptone in question. The precipitation of the respective amino-acids, or the appearance of the colour reaction, announces the presence of the decomposing agent.

Still more conclusive results are obtained if combinations of a known structure—for instance, polypeptides, in the building up of which the above-mentioned amino-acids take active part—be chosen for the experiment. Or one may follow the decomposition in a polariscope tube. A certain quantity of

[5] This is the case, for instance, with tryptophane.

the expressed juice is mixed with a measured solution of an optically active polypeptide of known composition. The mixture is poured into a polariscope tube and the rotation for the solution is ascertained as quickly as possible. If one then determines the rotation from time to time, an insight into the nature of the decomposition is acquired. Instead of optically active polypeptides we can employ racemic bodies. The latter are optically inactive, because they consist of two halves equally strong as regards their respective rotations in opposite directions. The peptolytic ferments generally decompose only such polypeptides as are built up out of the optically active amino-acids as they are found in nature. If we have to deal with a racemic polypeptide, of which one-half complies with this condition, then this part is reduced to its component parts, and we are left with the other half of the racemic body, which consists of amino-acids not found in nature. We recognize this asymmetric splitting through the fact that the original optically inactive mixture becomes optically active.

An example may convey a clear idea of these conditions. In nature we meet the amino-acids *l-leucin* and *d-alanin,* while *d-leucin* and *l-alanin* have never yet been found amongst the products of reduction of the proteins. If we allow peptolytic ferments to act on the racemic bodies *d-alanyl*—

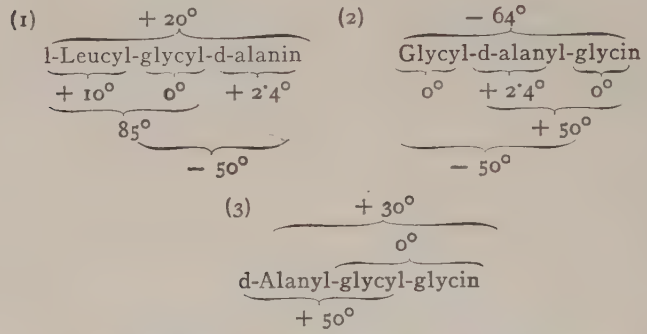
l-leucin + l-alanyl—d-leucin, then we obtain the amino-acids *l-leucin* and *d-alanin*, and are left with the compound *l-alanyl—d-leucin*. This is optically active.

Most interesting results are obtained when optically active polypeptides are chosen for examination in the building up of which several amino-acids take part. As in these bodies the rotation of every possible reduction stage is well known, it is easy to find out, in the most exact and unequivocal manner, at what particular stage the peptolytic ferment of a particular tissue attacks the substrate employed. We have thus a means at hand of comparing ferments of different origins, together with the possibility of recognizing, in the most exact way, all the specifically active peptolytic ferments. Further development of this field of research, by the use of the greatest variety of substrates from all kinds of substances, is required, in order to give an answer to the question of the peculiarities of certain kinds of cells in many directions. It will be possible in future to recognize certain cells by the manner in which they reduce substrates, the synthesis of which, as a matter of course, must be previously fully known to us.

An example will make clear this method of studying cell ferments.[6] The subjoined scheme supplies

[6] Here we have an enormous field, promising very fruitful results with respect to the most varied problems connected

information on the power of rotation of three polypeptides composed of three amino-acids. At the same time the optical relations of the individual decomposites are given.



The explanation of our example (3) illustrates the others as well. The tripeptide d-alanyl-glycyl-glycin rotates + 30°. If glycin (=glycocoll) were first split off by a ferment, then the dipeptide d-alanyl-glycin (see p. 53 (1)) would appear. The rotation of the solution would rise towards the right, because d-alanyl-glycin turns further to the right than the original material. If, on the contrary, d-alanin were set free first, then the rotation would soon decrease to 0°, as the resulting dipeptide glycyl-glycin is optically inactive (see p. 53 (2)).

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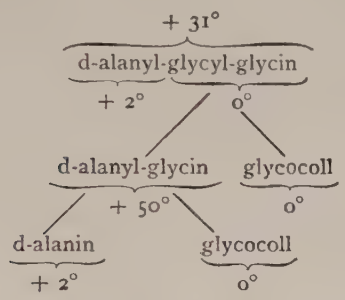
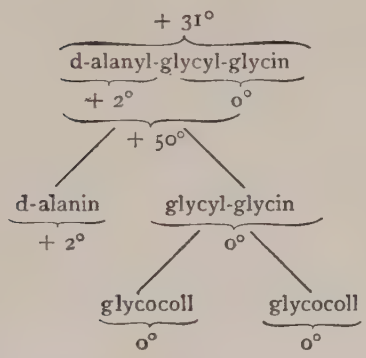
with the chemistry of albumen, with studies in immunity, with bacteriology, and so on, but which fails simply through lack of means for the upkeep of a small army of capable young chemists.

(1)



(2)



Finally, we may, for the purpose of tracing peptolytic ferments in tissues, inject into the tissues peptones and polypeptides, which contain sparingly soluble amino-acids, and observe directly whether any amino-acids are set free.

In all these experiments the co-operation of microorganisms was most carefully excluded, and there can be no doubt that these ferments belong to the tissues themselves. The same holds for ferments

that act on fats, carbohydrates, nucleo-proteids, nucleic-acids, phosphatides, and so on. Everything points to the fact that the cell has agents at its disposal which render it capable of splitting up, into their simplest units, all the complicated substances which are brought to it, or which it itself builds up. In favour of such a view, we may more particularly cite, besides the direct proof of the existence of ferments, the observation that in the metabolism of the cell all the units, out of which the complicated nutritive substances and the components of the cells are built up, are found to occur.

At the present time there is no doubt that an important part of the metabolic processes of the cells is furthered by ferments. In general, we may say that complicated substances are hydrolytically reduced in stages until the simplest structural units are formed. Once the latter appear, then the further reduction continues in stages, through various intermediate products, right down to the end-products of metabolism, or else the resulting products of decomposition form the starting-point for new syntheses. From these products very varied links are forged between very different groups of substances.

It is thus proved that, in a certain sense, each separate cell of the body is capable of digestion. This holds particularly for the white and red blood corpuscles; even the platelets are able to produce hydro-

lytic decompositions. The blood plasma is unable, either in the majority of animals or in man, to produce decomposition of albumens, peptones, and polypeptides, at least not in any degree which can be demonstrated by available methods. The capacity for decomposing fats is also apparently absent in most cases. On the other hand, we often meet with assertions that the blood always has a diastatic action, *i.e.*, the capacity for splitting complicated carbohydrates. Under normal conditions the blood plasma does not generally seem to be constructed for the reduction of complicated substances. Only in the case of guinea-pigs do we find conditions that are undoubtedly exceptional; here the blood plasma shows other properties, and even under normal conditions can partly break down polypeptides which are not in the least acted upon by the blood plasma of other animals. The cause of this peculiar behaviour of the plasma in guinea-pigs cannot yet be explained. That the blood plasma in general is lacking in digestive powers must obviously be construed in the sense that, under normal conditions, substances which are out of harmony with the plasma, and require a quick chemical reduction, never have access to the blood.

As soon as these observations had been made it become possible to study the question, whether the blood plasma exhibits new properties in cases where substances that are out of harmony with the plasma,

and particularly with the body, find their way into the plasma of an organism by any other way than through the intestinal canal. The order of these experiments was of the following character :—

In the first place we determined the composition of the blood plasma, or of the serum, in an animal, in regard to the proteolytic and peptolytic ferments it contained under normal conditions, that is to say, when the nourishment is normal. The manner in which this is done is as follows : 10 c.c. of blood is taken from the animal under experiment, for instance, from a dog, from the vena jugularis externa or from a vein of the leg. Either this is left to clot of its own accord, so as to separate out the serum ; or else 0·1 gr. of ammonium oxalate is added to the test-tube containing the blood, so as to prevent it from clotting. The form-elements are centrifuged out, and the clear plasma can then be withdrawn easily by means of a pipette. In both cases—serum and plasma—we must test for the absence of hæmoglobin, for, if it is present, then the red blood corpuscles have been broken up, in which case we may be quite certain that the ferments belonging to the red corpuscles have passed into the fluid derived from the blood. Only serum and plasma which are *absolutely free from hæmoglobin* must be used for these experiments. To a measured quantity of serum or plasma a certain quantity, in cubic centimetres, of

an albumen, peptone, or polypeptide solution, having a known composition in regard to substrates, is added; a polariscope tube is filled with the mixture, and the rotation is quickly ascertained by means of a good polariscope. The tube is then placed in an incubator, and from time to time the angle of rotation is again noted. To avoid mistakes another tube is filled with the same quantity of plasma or serum, and normal salt solution is added in the same quantity as the substrate solution employed; this mixture is then observed in the polariscope under the same conditions as the former. Finally, another test, with the substrate solution alone, is arranged in the same way. It is further essential to add to the mixture a measured quantity of a phosphate mixture for the purpose of preventing the action of the ferment from being in any way influenced by changes in the reaction of the mixture. To prevent cooling of the polariscope tube its jacket is filled with water at 37° C., or an incubator is used, which can be fitted to the polariscope (see below, the technique of the optical method). Decomposition of proteins or peptones could never be observed in these experiments, so long as the blood was taken from healthy, normally fed animals.

We now take the animal under observation, *i.e.*, the animal whose plasma or serum we are investigating, and introduce selected substances directly

into the organism, so as artificially to avoid the disintegrating action of the ferments of the intestines. These substances are injected either subcutaneously, or into the abdominal cavity, or else intravenously. After a certain lapse of time blood is extracted, and its serum, or plasma, is treated exactly in the same way as we have described above.

The first experiments were made with dogs and rabbits. White of egg, or horse blood-serum, was introduced parenterally into these animals, that is to say, avoiding the intestinal canal; tests were then made to see whether the plasma of the animals under experiment either decomposed certain polypeptides, or whether it decomposed them quicker than the plasma of the same animal did before the injection of the disharmonious substance. The very first experiments gave a positive result. It was found that the contents of the blood increased in peptolytic ferments. In a further experiment the substance used for the injections was silk-peptone. It was found that the serum of normal rabbits did not reduce this peptone at all, the angle of rotation of the mixture of plasma + peptone remaining constant. But if silk-peptone be injected into an animal, and the serum of the latter be then brought into contact with this peptone, then, if we take a rapid reading of the rotation in a polariscope, we find that the initial rotation alters in the course of time.

Experiments were then made with gliadin and with peptones obtained from gelatine, from edestin, and from casein. Edestin and casein were also injected by themselves. In all cases the result was the same. When substances, that are out of harmony with the plasma, are introduced into the plasma or serum of a given animal, we always find developed a special power of decomposing bodies belonging to the protein series, especially protein itself and its peptones. A specific activity of the injected substrates could be traced only so far as that, after the injection of proteins and peptones, ferments appeared in the plasma which were able to chemically reduce the derivatives of this group, but not, for instance, fats or carbohydrates. Conversely, no splitting of protein could be traced after injections of fats, of carbohydrates, or of amino-acids. On the other hand, after injection of a particular protein, or of a particular peptone mixture derived from a definite protein, not only were the injected substances decomposed by the plasma, but the decomposition extended to the whole group of proteins and their nearest derivatives.

That the process actually depends upon the presence of ferments can be proved in two ways. In the first place the splitting of a particular peptone solution, by the plasma of suitably prepared animals, was compared with the action of extracted yeast

juice on the same peptone. It was possible to show that the decomposition in both cases was very similar, *i.e.*, that the initial rotation varied in the same direction and to the same extent, whether the plasma of specially treated animals was used, or the active extract of yeast.

The following experiment proved, with exceptional clearness, that the plasma of an animal, when specially treated, actually reduces proteins. The plasma was mixed with gelatine or with white of egg, and the mixture was placed in a dialysation tube. Very shortly the presence of peptones could be demonstrated in the outer fluid—distilled water being chosen for this purpose—by means of the biuret reaction. But when plasma of normal animals was mixed with albuminous bodies and placed in a dialysation tube, no substances giving the biuret reactions could be traced in the outer fluid, even after standing for many days. Finally, it has been proved quite recently that, by mixing the plasma or serum of specially treated animals with albumen, the nitrogenous contents of the outer fluid increase to a considerably greater extent than when the plasma of normal animals and albumen are mixed together. In the last case the increase of nitrogen in the outer fluid is no greater than when the same quantity of plasma is brought into the dialysation tube by itself, *i.e.*, without addition of albumen. It must be understood

that, in this experiment, the albumen has to be previously freed from all nitrogenous and crystalloid admixtures by dialysis or by boiling.

When the plasma of specially treated animals, which experiment showed to be active, that is, to split up proteins and peptones, was raised to the temperature of 60° C. it became *inactive, i.e.*, its decomposing action could no longer be demonstrated.

The facts we have enumerated have been repeatedly verified by numerous experiments. In all these experiments on specially prepared plasma or serum control experiments have, of course, been carried out, on the one hand with peptone solutions alone, on the other hand with the plasma alone. Further, the serum, or plasma, was made inactive each time, for the purpose of avoiding any error, by raising the temperature to 60° C. Finally it was shown, by the use of the dialysation method, that the observations made by means of the so-called optical method were absolutely correct. It may also be mentioned that iodized albuminous substances were also injected, and that no splitting action of the blood plasma could be produced in this case. We know, from other experiments, that iodized albuminous substances are decomposed with difficulty or not at all. It seems likely that they are so intensely disharmonious with the body that the organism, even though armed with the proper ferments, can find no

point of attack from which to initiate their decomposition.

Some examples which, in the form of curves, represent the results obtained from the disintegration of mixtures of plasma, or serum, with some substrate (albumen or peptone), may serve to illustrate the above statements.

(1) A dog, whose serum did not decompose peptones, was given 0·5 gr. of casein by means of a subcutaneous injection on November 25 and 29 and December 4. The blood used in the experiment below was withdrawn on December 6. The polariscope tube was filled with a mixture of 0·5 c.c. of serum, 0·5 c.c. of silk-peptone solution (10 per cent.), and 7 c.c. of normal salt solution (see fig. 1).

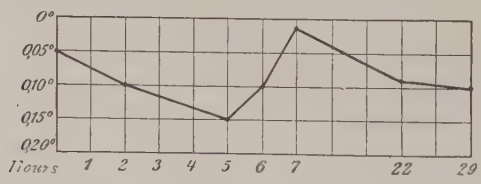


FIG. 1.

(2) A dog was given repeated subcutaneous injections of crystallized albumen obtained from seeds of the gourd. The last injection was made on December 8, 8 gr. of albumen being injected. The serum was examined on the following day. For this experiment 1 c.c. of serum was mixed with 0·5 c.c.

of a 10 per cent. gelatine-peptone solution, and 2·5 c.c. of normal salt solution (see fig. 2).

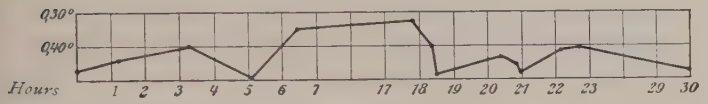


FIG. 2.

(3) The dog in this experiment was given on October 18, 3 c.c. of a 10 per cent. silk-peptone solution by subcutaneous injection. On October 21 blood was taken. The serum split up both silk-peptone (curve *a* in fig. 3), and gelatine (curve *c* in fig. 3). At a temperature of 60° C. the serum became inactive (curve *b* in fig. 3).

We may point out here that we thought it possible, at first, that the phenomena observed by us might have some connection with what is called anaphylaxy, or supersensibility.[7] By this we understand the

[7] Hermann Pfeiffer, of Graz, at about the same time as, and independently of, ourselves, has demonstrated the existence of proteolytic ferments in the blood plasma of sensitized animals, after we had already established the fact of the appearance of peptolytic ferments subsequent to the introduction into the blood of disharmonious derivatives of albumen, and had in this way systematically treated the whole problem. The first experiments were made with albumen. They have since been abandoned, because the results of an alteration in rotation seemed particularly ambiguous in cases where the serum of animals, treated previously with albumen, was brought into contact with albumen or peptone. For this reason polypeptides are preferable for reactions on ferments, as being compounds whose exact structure is known to us.

extraordinary property possessed by the animal organism of responding with certain typical symptoms to a second injection of the same material as was used in the first injection. A certain time elapses—in the case of a guinea-pig, about fifteen to twenty days—before this state is overcome.

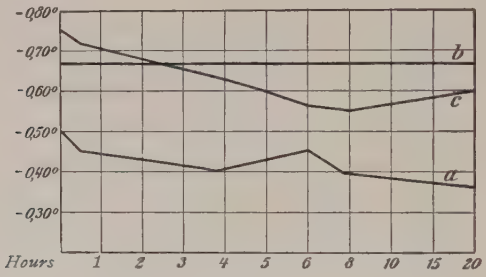


FIG. 3.

(*a*) 1 c.c. serum.
0·5 c.c. of a 10 per cent. silk-peptone solution.
5 c.c. normal salt solution.
(*b*) 1 c.c. serum at a temperature of 60°.
0·5 c.c. of a 10 per cent. silk-peptone solution.
5 c.c. normal salt solution.
(*c*) 1 c.c. serum.
1 c.c. of a 1 per cent. gelatine solution.
4·5 c.c. normal salt solution.

Cramp can be observed within different groups of muscles, as well as a sudden fall of temperature, &c. Peptones also can be demonstrated in the blood after reinjection of the original protein. Various authors have supposed that anaphylaxy is directly connected with the production of derivatives of proteins, particularly peptones, without, however, having

succeeded in supplying definite proofs for such a view. It is only recently that experiments have been made, by means of injections of peptones and derivatives of amino-acids, especially of amines, with a view to producing phenomena resembling those of an anaphylactic shock. It is difficult to decide with any certainty what part is played, by the ferments we have observed, in the setting up of anaphylaxy. Several facts run counter to the supposition of a direct relation between the presence of active ferments and the particular substrate against which they are directed. It has been proved, beyond doubt, that these ferments exist in the blood at a time when the anaphylactic shock cannot yet be produced by a second injection of the same material as was used in the first case. Further, it has already been pointed out that these ferments are specific only in respect of the group of substances which are used for the injection, but not for the particular body that has been introduced. To produce the shock, on the contrary, the substrate, with which the animal under experiment was rendered sensitive, must be present. A certain importance, in regard to the setting up of the state of shock, may be attached to the power possessed by the plasma of decomposing albumen ; as is shown by an observation which was made by Hermann Pfeiffer and confirmed by ourselves, according to which the proteolysis in the plasma disappears during the

moments following the anaphylactic shock, *i.e.*, during so-called antianaphylaxy—a state in which the animal becomes absolutely insensitive towards further injection.

If we summarize all the results obtained up to date, we arrive at the conclusion that our observations with regard to the appearance of ferments in the blood plasma, after injection of disharmonious proteins and peptones, undoubtedly stand in some kind of relation to anaphylaxy. The special significance of these ferments, however, remains uncertain. It would appear possible that these ferments acquire some special properties in the course of time, and then, by decomposition of the second dose of albumen, give rise to derivatives of a highly specialized nature and activities.[8]

There are many other possibilities to be considered. The decomposition may not necessarily take place only in the blood. Our method has at present only demonstrated the appearance of ferments in the plasma or serum, and that could only be done because the ferments, which we find after parenteral introduction of proteins and peptones, cannot normally be traced in the blood plasma of certain animals. It is not unlikely that, after the introduction

[8] Other substrates, which are also decomposed, may not give the same derivatives, in which case a specific activity of the material first injected would be assured.

of substances out of harmony with the species, new properties appear also in the cells of the body, and that the latter undertake likewise the decomposition of these disharmonious substances. In a certain sense each individual cell would act in the presence of the disharmonious material exactly in the same way as an unicellular organism, and fight them to the extent with which it is provided with the necessary weapons —the ferments—that enable it to make a successful attack on the substrate. Like primitive organisms, too, it is able to protect itself against the penetration of these substrates by means of the constitution and quality of its walls, and so to wait until the modification of the substances has been effected elsewhere to such a degree, that all their disharmonious properties have disappeared, and only an indifferent product remains.

Finally, it may be that the whole problem of anaphylaxy will not be resolved by purely chemical considerations only. Why should not disturbances originating from dislocations of osmotic equilibrium, or activities of special ions, be taken into account, and associated with the other observed phenomena. (*Cf.* on this point Lit. 13.)

The more widely the limits of these problems are extended, the more probable does it become that the experimental testing of all possibilities will put us on the proper road for an explanation of the phenomena

observed. Surely it would be absurd to limit the study of anaphylaxy only to a study of the behaviour of the blood; for it is more than likely that it is the cells of the body which ultimately play the chief part in the appearance of anaphylaxy. The behaviour of the blood plasma is possibly only a reflection of the defensive measures adopted by the cells of the body; while, in any given case, it may be only a special type of cell that has to be considered.

Special interest attaches to the proof of how the organism reacts when blood of its own kind, or from another animal species, is introduced into its circulation. In the latter case ferments appeared in the plasma, which decomposed albumens and peptones. If harmonious blood were chosen from an animal of the same race, no reaction whatever was noticed when it was transmitted directly, *i.e.*, without leaving the blood-vessels. When, on the contrary, blood which belonged to an entirely different race was introduced into a dog, then a decomposition could be demonstrated within the circulation.

Against the results thus obtained one might raise the objection that the appearance in the circulation of active reducing ferments would give rise to enormous disturbances, because even those albuminous bodies that are in harmony with the plasma are liable to be attacked by them. But this is evidently not the case, since the plasma, though containing an

active ferment, retains its initial angle of rotation; and it is only in very exceptional cases that dialysis shows the presence, in the outer fluid, of substances that give the biuret reaction. It is only after proteins or peptones are added to the plasma, that the activity of the ferments first manifests itself.

How can we explain a behaviour that is, a priori, so peculiar? There are already, before the addition of the proteins or peptones, large quantities of albumen in the plasma in the presence of an active ferment. We must always remember, in this connection, that the ferments are directed, in a more or less explicitly specific manner, against certain substrates. A slight alteration in structure and configuration suffices to remove a substrate from the influence of a given ferment. Just as the ferments themselves are first transformed into their active form by means of a special agent, so, without doubt, the substances in the blood and the cells which are presented to the ferments need special agents to bring them into a condition suitable for attack.

The substrates, too, are rendered active in a certain sense. The body defends its cells, and the substances contained in them, against disintegration by ferments by giving them a structure and configuration—it may be that their physical condition also plays a part—which are out of harmony with the ferments; and from this point of view we can understand why

the harmonious proteins of the plasma are not attacked by the ferments which circulate in the blood.

Finally, the question may be raised, why the decomposition of parenterally introduced proteins and peptones cannot be followed up directly, by observations on the rotating power of the plasma, without the addition of proteins or peptones. If the appearance of proteo- and peptolytic ferments in the plasma has the object of undertaking the decomposition of the substrates introduced into it, then we ought to be able to follow up the digestion—the decomposition—in the plasma itself. As a matter of fact it has been found possible to demonstrate, by means of intravenous introduction of large quantities of proteins and peptones, after the animals have been prepared by previous injections, that, when the blood is withdrawn immediately, not only has an alteration taken place in the original rotation of the plasma, to which nothing has been added, but also that peptones may be found in the outer fluid in the dialysation test. That this demonstration does not generally succeed—*i.e.*, that the decomposition of the substances that are out of harmony with the body cannot be followed up by means of observations on the plasma alone, without the addition of substrates—depends primarily upon the fact, that the injected substances suddenly become

very much diluted, and then probably pass straight into the lymph, and possibly also into the cells of the body. The optical method is not so exact as to permit us to establish very minute changes in rotation, and, even if it were possible to observe such rotations, it would be impossible to know for certain whether the fluctuations were not within the limits of errors of observation. Moreover, the decomposition undoubtedly proceeds quickly, so much so that we are really indebted to a lucky chance when we are able to follow up the decomposition of the injected matter in the plasma itself. These are the reasons why we have to prove the presence of the ferments by means of substrates, against which the respective ferments are directed. The substrate is the reactive for its corresponding ferment, and the decomposition of the former betrays the presence of the latter.

It may be remarked that the clear establishment of the presence of proteo- and peptolytic ferments in the blood plasma, after injection into the circulation of albuminous substances that are out of harmony with the body, has supplied a real explanation of the behaviour of parenterally injected proteins during metabolism. There is no longer any doubt that they are made use of, that is, that they are utilized in the metabolism of the cells of the body, so far as experience has shown decomposition to be

possible. Different observers (Lit. 4, 8, 10, 11, 12, 16, 17, 18, 19), who have instituted experiments on metabolism subsequent to parenteral introduction of proteins, have suggested that decomposition by means of ferments takes place outside the intestines. This is most clearly stated by Heilner. This suggestion, however, was only proved by the direct demonstration of the ferments by means of the experiments and methods we have described.

The positive knowledge that it is possible to induce a splitting activity in the blood plasma of animals, the plasma of which is otherwise unable to decompose albuminous substances, by means of parenteral injections of these substances, led of itself to the problem whether analogous phenomena appear when other substances, which are out of harmony with the body and the plasma, but do not belong to the albumens, are used in such injections. We began with the parenteral introduction of disharmonious forms of sugar. In the first place it was ascertained that the plasma or serum of dogs is unable to split up cane sugar. If blood serum, or blood plasma, of a dog be brought into a solution of cane sugar, it can easily be demonstrated, by means of analytical methods, that the cane sugar does not undergo any alteration. Certainly no decomposition takes place. The contents of the blood plasma are not increased in respect of reduced substances. If,

however, in this experiment we use the blood plasma, or serum, of a dog to which cane sugar has been administered as an injection, either subcutaneously or directly into the circulation, then, on bringing this plasma and cane sugar together, we observe that the reducing potentialities of the mixture are considerably increased. Simultaneously, it is possible to show that the quantity of the admixed cane sugar diminishes.

These experiments give very positive results when the splitting action of the plasma is investigated with the aid of the optical method. In this case plasma is taken from a normal dog in a certain quantity, and a known amount of cane sugar solution is added; a polarization tube is filled with the mixture, and the rotation of the latter is ascertained. The readings of the polariscope are taken from time to time, and the tube is kept during the intervals in an incubator at 37° C. It is found that the initial rotation keeps constant.

Now, if an injection of cane sugar is made into the circulation of the same dog from which the plasma was taken, it may be demonstrated after a very short time that its plasma is now capable of breaking up cane sugar. The strong rotation to the right, which we observe at first, decreases continuously. It approaches zero, and finally, passing zero, it travels to the left. We obtain eventually a left-handed

rotation, the cane sugar being converted into invert sugar. The latter consists of one molecule of grape sugar and one molecule of fruit sugar; that is, of the units of the disaccharide, cane sugar. Since the fruit sugar turns more to the left than the grape sugar does to the right a final rotation results to the left. Many observations point to the fact that, at the same time, part of the products of the decomposition suffer further disintegration.

Parenteral introduction of cane sugar does not always succeed in effecting the appearance of invertin in the blood plasma. Obviously, the time during which the disharmonious substance remains in the blood plays an important part in the formation of the defensive ferments. The cane sugar is very quickly excreted through the kidneys.[9]

The following examples will give an idea of the results of these experiments :—

(1) A dog was given subcutaneous injections of cane sugar (5 gr. at a time) on October 22 and 23. The blood taken on October 24 was used for testing

[9] It has been pointed out in original communications that, in the parenteral introduction of carbohydrates, no such regular results can be obtained as is the case with proteins; for the latter remain longer in the circulation, and are not usually excreted by the kidneys. The organism is, in this case, directly dependent on the composition of the products for its freedom from disharmonious substances. In the case of cane sugar, the kidneys are able of themselves to deal with the disharmonious compound.

the behaviour of the serum towards cane sugar. To 1 c.c. of serum was added 1 c.c. of a 10 per cent. solution of cane sugar and 5 c.c. of normal salt solution. The initial rotation of the mixture was + 0·45°. At the end of the experiment the rotation had sunk to - 0·50° (see fig. 4).

(2) Blood was taken from a dog before the parenteral introduction of cane sugar, and the behaviour of

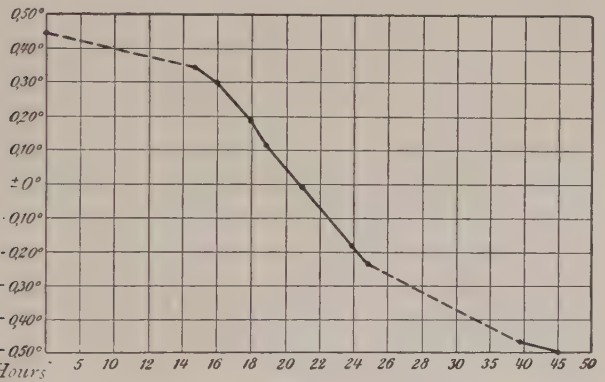


FIG. 4.

the serum towards this disaccharide was ascertained. Decomposition did not take place (curve 1 in fig. 5). Then 10 c.c. of a 5 per cent. solution of cane sugar were given to the animal by intravenous injection. The sample of blood, taken fifteen minutes after the injection, already showed hydrolysis of the cane sugar that had been added (curve 2 in fig. 5). For the purpose of control the rotation of the serum without the

addition of cane sugar was noted (curves *A* and *B* in fig. 5). The arrangement of the experiment is shown in the following summary :—

(1) 0·5 c.c. serum (blood taken before the injection of cane sugar).

0·5 c.c. of a 5 per cent. solution of cane sugar.

7 c.c. normal salt solution.

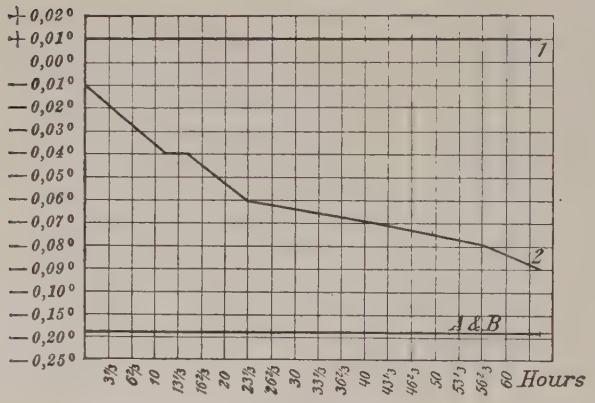


FIG. 5.

(2) 0·5 c.c. serum (blood taken fifteen minutes after intravenous injection of a solution of cane sugar).

0·5 c.c. of a 5 per cent. solution of cane sugar.

7 c.c. normal salt solution.

A and *B*. 0·5 c.c. serum.

7·5 c.c. normal salt solution.

(3) Further experiments were undertaken for the

purpose of studying the question, how long after the actual parenteral introduction of cane sugar the presence of invertin in the blood serum may be demonstrated. After a single subcutaneous injection of cane sugar the power of decomposing this disaccharide was still traceable at the end of fourteen days (curve I in fig. 6). In a dog, which received a

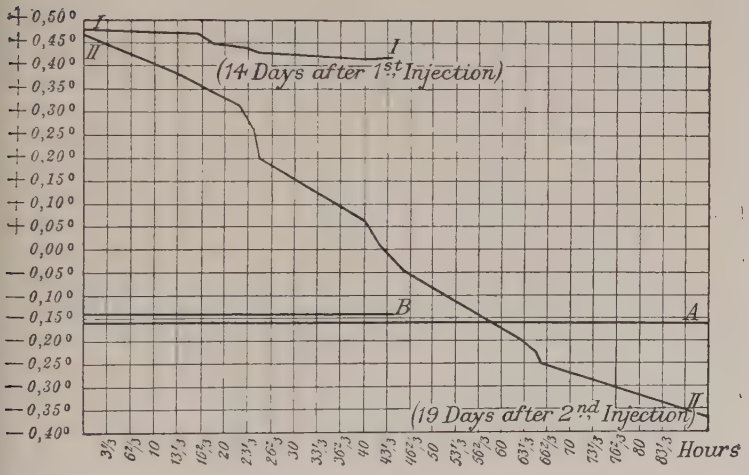


FIG. 6.

subcutaneous injection of cane sugar on two occasions, it was still possible to bring about an energetic splitting of this disaccharide with blood serum after nineteen days (curve II in fig. 6). The property once acquired does not therefore disappear at once. The individual experiments were conducted with the

following quantities of serum and cane sugar solution :—

(1) 0·5 c.c. serum (blood taken fourteen days after injection of cane sugar).
0·5 c.c. of a 10 per cent. solution of cane sugar.
7 c.c. normal salt solution.

(2) 0·5 c.c. serum (blood taken nineteen days after the second injection of cane sugar).
0·5 c.c. of a 10 per cent. solution of cane sugar.
7 c.c. normal salt solution.

Control Test.

A and *B*. 0·5 c.c. serum.
7·5 c.c. normal salt solution.

These results, without our knowing it, confirmed experiments which Weinland had made before us. He had already been able to show that the blood plasma of a dog is able to split up cane sugar; that is to say, it contains invertin as soon as cane sugar is parenterally introduced. These experiments were then later extended to other kinds of sugar, and especially to milk sugar. It was possible to show that the latter also undergoes alteration, but it seems that here, alongside of a hydrolysis, a decomposition takes place in another direction.

Very extraordinary is the observation that, after the introduction of soluble starch, and also of milk sugar, the blood plasma or serum is able to decompose

cane sugar. It seems as if here also, after the introduction of a disharmonious kind of sugar, ferments appear which are not exclusively directed against the carbohydrate injected. Further, the ability of the organism to supply ferments seems to have limits, because, after an injection with raffinose, no definite reaction could be traced. Very likely this material is too markedly disharmonious with the cells of the body.

It is interesting to note that ferments contained in the plasma persist for a long time after the introduction of disharmonious substances, whether they be products of the albumen order or of the carbohydrates. The splitting activity of the plasma could still be clearly traced in individual cases up to three weeks after the injection. Important, too, is the fact that, after an intravenous injection of cane sugar, invertin could be demonstrated in the blood plasma within a quarter of an hour. When albuminous substances were introduced subcutaneously, then three to four days elapsed before the formation of the ferments attained its full value. After intravenous injections they manifest themselves within twenty-four hours. The fact that there are individual differences is also important. Moreover, the appearance of the defensive ferments is very much delayed after the introduction in large quantities of substances out of harmony with the blood.

Finally, the behaviour of fatty products was tested. In this case difficulties of method were encountered at first. The experiment made to ascertain the decomposition of fat in the blood, by simple titration of the acids produced, failed entirely. The question, whether, after the introduction of fats that are out of harmony with the body and the plasma, an increase follows of the amount of lipase in the plasma, could only be attacked after Michaelis and Rona had selected the alteration of surface-tension during dissociation as the basis of a method for the study of the decomposition of fats. The fats belong to a group of substances that are strongly surface-active, while the products of disintegration produced by their decomposition, such as alcohol and fatty acids, possess no marked influence over surface-tension. If plasma of a normal animal be mixed with any kind of fat, such as tributyrin, and the mixture be allowed to flow from a capillary tube, a certain number of drops escape in a given time. But if any fat gets into the animal's circulation by whatever means, then the number of drops escaping through the capillary tube decreases.

As far as we can judge from the experience gained up to the present, it seems that the conditions met with in fats are much more complicated than in the case of proteins and polysaccharides. This experience tells us that while, under normal conditions,

proteins of a definite kind, and in obviously definite quantities, are always circulating in the blood, and the amount of carbohydrates also varies only within narrow limits, the fats behave quite differently. The amount of fats in the plasma varies within a wide range. After a meal rich in fat we find so much fat in the blood plasma that it may be seen with the naked eye; and, if we let the plasma stand, the fat separates out directly and appears as a layer on the surface of the plasma. A short time after the meal the fat disappears again from the blood. It is transmitted to the different cells of the body, and is there used up, transformed, or even directly stored as reserve material. It seems that the blood, with every increase in the amount of fat, responds with an increase of lipase. From the point of view we have laid down the excess of fat has to be considered as being out of harmony with the plasma. Only in an animal whose stomach is completely empty do we find no, or very little, capacity for splitting fats. After a meal rich in fat active lipase can be demonstrated in the blood. It can also be shown that during a more or less prolonged hunger period the splitting power of the blood increases. This corresponds with the experience that, during fasting, an active transportation of material is going on. In many cases of fasting large quantities of fat could be shown to be present in the blood. If a fat be introduced that is

out of harmony with the species, we find in the plasma an exceedingly high capacity for splitting fats.

In the case of fats we meet with some difficulties when we try to introduce, into the circulation, fats that have not been modified so as to be in harmony with the plasma. If they are injected subcutaneously they remain at the point of injection for a long while, and are probably only transported further after the actual splitting has begun. In cases of intravenous injection one runs the risk of killing the animal, owing to fatty embolisms. The introduction, into the blood, of a fat that is out of harmony with the species could only be effected for the first time after an old experiment of J. Munk had been made use of, namely, to gorge an animal with an excessive quantity of fat, so that it may easily be demonstrated in the tissues and, naturally, also in the blood. We fed in this way on large quantities of rapeseed oil and mutton suet, and then found in the plasma a very strongly marked capacity for splitting fats. We may mention here, also, that the same effects may be produced, with proteins and peptones, and also with carbohydrates, as in the case of parenteral injection, if an excess of these substances is forced through the intestines by flooding the intestinal canal with the particular nutriment. We would also emphasize the fact that a state of anaphylaxy may be successfully set up by this means. If we

introduce into an animal a large amount of white of egg, there is no doubt that unaltered protein passes into the circulation. It is also possible that peptones are absorbed, which still have the specific structure of the white of egg. This transfer may be ascertained by the so-called biological reactions, precipitin reaction, &c., but chiefly and most exactly by demonstration of the existence of peptolytic ferments in the circulation. If, after a certain interval, white of egg is once more introduced by either parenteral or enteral means—in the latter case the supply must be very copious—the state of shock is obtained.[10]

Seeing that harmonious fats, as we have already mentioned, also produce in the circulation an increased power of splitting fats, it is rather difficult to determine whether disharmonious fatty products give rise to a definite specific activity. Further experiments will be needed to determine this point.

Finally, we have also made injections of nucleoproteids, nucleins, and nucleic acids, introducing them into the organism in such a way as to prevent them passing through the intestine. We found that, after introduction of these bodies, ferments appear in the blood plasma in increased quantity, and quickly reduce these substances (see also Lit. 21). Moreover, it could be shown that it was possible, by means of

[10] Enteral sensitization and subsequent enteral shock have been successfully accomplished by us on two occasions only.

certain nucleo-proteids as well as nucleins, to excite anaphylactic phenomena of a very specific character. Experiments on guinea-pigs, which were made in collaboration with Kashiwado, showed that a second injection of the same substance as was used in the first instance produced specific cramps of the muscles of the neck and of the jaw. Moreover, an increased peristalsis was regularly present, the animals excreting fæces continuously. Symptoms of lameness soon set in, and a marked fall of temperature always took place. We injected, for instance, nucleo-proteids, nuclein substances which had been obtained from the thymus, and finally nucleo-proteids from the blood corpuscles of a goose; the reaction was in all cases a strictly specific one. In the case of nucleic acids we were unable to get definite results, and it would appear that these cannot produce anaphylactic phenomena. It may be that, in the nucleo-proteids and in the nucleins, their albuminous components are the deciding factor. A systematic study of the nuclear structure of the various cells of the same individual may enable us to determine the question, whether albumens that are in harmony with the nucleus take part in its construction, or whether the nucleus plays a part, in the cellular metabolism, which is repeated in an identical manner in the various cells of the same individual.

So long as purely chemical research is unable to

answer questions concerning the finer structure of the cell elements, we are bound to resort to indirect methods. The latter have, in a relatively short time, opened up a vast field, and discovered views of the widest interest in regard to every kind of cellular process. It is the duty of the future to follow up with exact methods all the observations that have been made, and to replace with known quantities the many unknowns with which our present-day methods have still to reckon.

If we sum up all the phenomena observed, we get the following picture: By introducing substances which are out of harmony with the species, and more particularly with the plasma, we bring into the organism bodies which are, in their whole structure, absolutely disharmonious with the cells of the body. No alteration has as yet taken place. In order that these substances may be utilized by the cells of the body, the products that are suitable for the organism must be so far decomposed as to entirely lose their specific character. This decomposition is effected by ferments, and is certainly initiated very quickly, for the disharmony with the blood or plasma and the body may extend also to the cells, and have an injurious effect on them. During the decomposition of these substances products arise at first which are, in part at least, definitely disharmonious with the

organism, and these may be equally injurious under certain conditions. If these products of a gradual decomposition constantly appear only in small quantities and further decomposition is very rapid, then the injury will be but trifling and transitory. When, on the contrary, a large quantity of these products of decomposition suddenly appear, they can produce serious disturbances by their combined action. In these processes it is not only their chemical nature, their structure and configuration, that is of importance; we have to remember that, during the decomposition of colloid substances, products arise which exert an influence upon the osmotic pressure, and may, in consequence, disturb the existing equilibrium. What we observe in the plasma also takes place, as we have already emphasized, in the interior of the cells, and probably in a similar way. It may be pointed out here that, when bodies of a simple constitution, such as crystalloids, are introduced, the organism is able to defend itself, not only by decomposing such disharmonious substances, but also by excreting a part of them, at any rate, through the kidneys. The same method of defence may be resorted to when, during the decomposition of complicated substances, simpler particles are produced. In this case the excretion accelerates the ejection of the disharmonious substance from the body. It is true that, in doing this, the organism loses valuable

fuel on the one hand, and certain constructive units on the other.

Many observations point to the fact that parenterally introduced substances, in so far as they can be modified, are utilized by the organism; that is, they serve as nourishment. The digestion, which would otherwise take place in the intestine and prevent the passage of disharmonious material into the body, is performed by the blood.

It is an open question from what source these ferments, which we are going to call defensive ferments, take their origin. Many facts accord with the suggestion that the leucocytes play a part in this connection (see also Lit. 23). They probably give off these ferments to the circulation. If so, we should then have in the blood plasma phenomena more or less analogous to those observed, for instance, by B. Friedrich Müller, during the dissolution of the fibrin that is excreted into the alveoli in cases of pneumonia. We see here numerous leucocytes penetrating into the solid exudate and dissolving it, after which an absorption of the products of decomposition begins, a kind of digestion taking place in the interior of the alveoli. Here also, as can be shown by special experiments, ferments can be demonstrated in the contents of the alveoli (in the expectorated sputum); and these ferments take their origin from the leucocytes. The old view, whereby the leucocytes take up

substances from the outside and digest them, must now be completed by the observation, that ferments can be given off to the exterior, and that therefore digestion may be accomplished outside the cell. We would like for the present to leave the question open, whether any importance can be ascribed, in this connection, to the white blood corpuscles generally, or to any special forms of these. We presume that the red blood corpuscles as well, and very likely also the blood platelets, play an important part in these processes. The presence of ferments in these cells must not, it is true, be unconditionally connected with the formation of defensive ferments, because it is clear that these cell elements must have means of reducing their nutriment to simpler molecules, and constructing their own bodies. In any case, it is extraordinary that, in these kinds of cells, there are such active ferments present, and in such large quantities. According to our experiments, the splitting processes in these cells take place much quicker than in the other cells of the body. It is certain that the red blood corpuscles have, besides the function of transporting the oxygen, other duties to fulfil in the economy of the organism. We further consider it quite possible that the same cells, which give off insufficiently harmonized products to the blood, also supply the ferments which are able to complete the decomposition in the circulation.

According to our observations, there is not the slightest doubt that the animal organism is not left without means of defence against disharmonious substances. If such products make their way into the body, the latter sends out defensive ferments that are directed against special kinds of substrates. Not only do they effect the destruction of the specific character of the parenterally introduced substance by means of an extensive decomposition, but they render possible the utilization of the products of the decomposition in the general metabolism. The reaction we have demonstrated enables us at any time to decide whether a certain substance is in harmony with the body or not. We have already emphasized the fact that we must distinguish not only substances that are in, or out of, harmony with the body, but also those which are in, or out of, harmony with the blood or its plasma, or again with the cells. We have already described how the intestine, with its ferments and those of its accessory glands, decomposes all disharmonious substances until an indifferent mixture of only the simplest units is left; and how then the cells of the gut-walls, and of the liver, carefully test the absorbed products for the absence, or transformation, of all substances that are out of harmony with the body and blood. Moreover, all the cells of the body take care that nothing shall pass from them into the circulation which has not attained a certain

grade of decomposition. For further protection, the lymph, with all its complicated arrangements, is interposed between the circulation and the cells of the body. Here everything is tested afresh; and nothing is let loose into the circulation that has not been rendered harmonious with the blood and its plasma. For ourselves, we have scarcely any doubt that the lymph system plays an important part in metabolism, along the lines we have indicated. Sometimes substances are reduced and converted into harmonious material; sometimes products of a definite type are built up. The lymph is, as we have pointed out before, to be considered as a sort of buffer between the cells of the blood and those of the body; as a neutral zone, in which everything is assimilated as far as possible.

If these views are correct, it should be possible to trace such substances as are in harmony with the body, but not with the blood and its plasma, by looking for definite ferments. It is quite conceivable that, in certain diseases, the cells only partially effect the decomposition of the nutritive material and of the constituents of the body, and that, to a certain extent, materials that are harmonious only with the cells are handed on to the lymph. The lymph, as already pointed out, would in many cases do its best to correct this failure by means of its cells, the leuco-

cytes, and its special organs, the lymphatic glands, and would attempt to decompose some of the disharmonious products before they reach the blood. In many cases, however, disharmonious material will get into the blood, and produce all kinds of disturbances.

We know of at least two conditions in which disharmonious substances undoubtedly circulate in the blood, namely, Bence Jones's albuminuria, and pregnancy. In the latter case it is certain that we are dealing with substances that are in harmony with the species, while in the first case there is a possibility that disharmonious substances are present. Bence Jones's albuminuria is nearly always found in conjunction with sarcoma of the bones. Whether the sarcoma is to be regarded as a harmonious tissue or not, we are not yet in a position to decide.

As to pregnancy, we know, thanks to the important observations of Schmorl, that the cells of the chorionic villi may be torn off, and be carried into the circulation. Veit has indeed proved that these cases are relatively frequent.[11] Weichardt, and later also Richard Freund, tried to connect the appearance of chorionic villi in the circulation with eclampsia. Weichardt was thinking of a dissolution of the cells—cytolysis—in which case toxic products are supposed to appear.

[11] *Cf.*, on this point, Hans Hinselmann, "Die angebliche, physiologische Schwangerschaftsthrombose von Gefässen der uterinen Plazentarstelle." Ferdinand Enke, Stuttgart, 1913.

To us these observations and views had this much value: that they directed our attention to the fact that, during pregnancy, the appearance of substances, that were in harmony with the species but not with the plasma, might be possible. If our view is correct, that the animal organism sets free ferments of a special kind, as soon as material that is in harmony with the species, but not with the plasma, passes into the blood, then it ought to be possible to demonstrate the existence of such ferments during pregnancy. As, however, the ferments, as we know from experience, disappear fourteen to twenty-one days after the actual introduction of disharmonious substances, it was scarcely possible to expect that defensive ferments should be met with during the whole period of pregnancy. One had to experiment with a great many cases, before striking that one in which dissolution of the cells of the villi had just taken place.

Experience, however, soon showed that the serum of pregnant individuals always contains defensive ferments which are directed against placenta albumen. We cannot, therefore, maintain that the tearing off of the epithelium of the chorionic villi is the only cause of the appearance of the defensive ferments. It must be remembered that even the mare exhibits, during pregnancy, defensive ferments which are directed against placenta albumen; yet the placenta of the mare is related to the circulation in

such a way as to exclude the possibility of the cells of the chorionic villi getting into the blood.

How can we then explain the existence of the defensive ferments during pregnancy? It can only be in very exceptional cases that they are produced by the invasion of morphological elements. In most cases it must be the result of the transfer of particular bodies—the constituents of particular cells, or the products of their decomposition. It may be, of course, that the extraordinarily active metabolic processes, which arise at the junction of the maternal and fœtal organisms, result in an insufficient reduction of many of the products of the cells of the placenta; the metabolism, in short, overreaching itself through its own rapidity. It is, however, also conceivable that the cells themselves break up easily.

The following view is probably the correct one. The organism of the mother has at its disposal, up to the appearance of pregnancy, a certain amount of cells of a certain kind, which all harmonize, in their metabolism, with each other. Now, with conception, comes the appearance of an entirely new kind of tissue, which has to perform particular duties. Although the impregnated egg and the developing placenta, with all its various cells, are in harmony with the species, nevertheless the metabolism of all these cells appears as something quite new and strange to the complex of cells composing the

organism of the mother. The blood probably receives substances—perhaps also secretions—which are out of harmony with the plasma, and remain so; and the time is too short for the blood to accustom itself entirely to these new kinds of substances. The placenta, according to this point of view, together with the fœtus, never settles down completely within the organism of the mother. With the expulsion of the placenta, in which process, again, ferments probably play a preparatory part, the ferments which are directed against its albumen disappear fairly quickly. It is, of course, quite possible that these placentally active ferments are conditioned by many different forces.

The view we have just discussed gives us an opportunity of demonstrating the initiation of the function of a particular organ. Were an organ to suddenly take up a particular function at a particular moment —say the delivery of a particular secretion—then it is quite conceivable that its activities would, at first, be disharmonious with the plasma. We might possibly expect something of this kind from the sexual glands at the onset of puberty; but experiments carried on in this direction upon animals in heat have not as yet given any certain result. On the other hand, the cessation of the functions of a particular organ might lead to the appearance of substances out of harmony with the blood; for these functions do not cease suddenly, and it may be that their gradual dis-

continuance leads to the appearance of insufficiently decomposed products. Involution, too, may be the cause of the formation of products that are out of harmony with the blood; in which connection we have in mind more especially the degeneration of the thymus, and the climacterium.

Many of our own researches, and those of various observers, have shown that, during the whole period of pregnancy, defensive ferments circulate in the blood, which are able to reduce placenta albumen. These ferments may be demonstrated within about eight days after impregnation. Their presence is, without any doubt, dependent upon the circulation of disharmonious substances originating in the placenta; since the defensive ferments disappear within fourteen to twenty-one days, when the relations of the placenta with the maternal organism have ceased.

Attempts have also been made to decompose placental tissues by means of the blood serum of the fœtus. No digestion could be induced; nor can the serum of pregnant individuals be made to attack the tissues of the fœtus. In any case these observations must be carefully followed up. It might be imagined, a priori, that there are developmental stages, in which the tissues of the fœtus are as yet so little differentiated, that they are still of a generalized character. Nor did umbilical blood

serum show any decomposition, when mixed with the serum of impregnated individuals. Had that been the case, then a very simple method for the diagnosis of pregnancy would have been found.

From the experience we have already gained, we may venture to say that the behaviour of blood serum towards coagulated placental tissue, or to placenta peptone, enables us to diagnose a state of pregnancy in the clearest possible way; or, expressed more correctly, to decide whether a placenta is in existence which is still in communication with the organism of the mother. A limitation is only necessary, because the defensive ferments can still be traced for a certain time after the expulsion of the placenta. For the practical sero-diagnosis of pregnancy this circumstance is of little importance, because the case under observation can be examined clinically. Normal non-pregnant individuals do not show any disintegration of the placental tissue.

We now had to decide the extremely important question, whether defensive ferments of a more general nature appear, when the organism contains other substances that are out of harmony with the plasma. This problem may be precisely expressed in the following manner: Does the serum of individuals, who suffer from infectious diseases, or from carcinoma, or who exhibit a disease of any other kind, decompose placental tissue? A priori, one was driven

to the conclusion that they would do so, because it had been noticed that, after the introduction of disharmonious substances into the circulation, not only do ferments appear which reduce the particular compound inoculated, but the defensive ferments produced attack many other substances of the same order. There was no production of strictly specific ferments, but only of those which are limited in their action to a particular class of compounds. And when substances that were in harmony with the body, but not with the plasma, were chosen for these experiments, no ferments appeared that were strictly specific.

We tested the sera of tuberculous individuals, of sufferers from carcinoma, of persons with salpingitis, and others, for their behaviour towards placental tissue, but in not a single case did decomposition take place. To our great surprise it appeared, that the animal organism only sets free strictly specific ferments, when particular cells are themselves giving off substances which are not in harmony with the plasma.

How can we explain this different mode of behaviour, according to whether we introduce such disharmonious substances, or whether the organism itself supplies them? There are various possibilities to be considered. In the first place the cell may give off the substances in question only in minute quantities, a condition which we are unable to imitate. Our methods of interference are

always brutal, and at once produce pathological conditions. We cannot imitate this method of supplying a substance in minute quantities, simply because we have no means of controlling the essential mechanism of regulation, owing to our ignorance of its nature. By our injections of disharmonious material we suddenly alter the composition of the blood, and injuriously affect the whole organism. It is, in this respect, most interesting that defensive ferments which only decompose cane sugar, can be obtained when, for instance, cane sugar is injected in very small quantities. As the quantity of cane sugar is increased then, very often, the blood serum is able to dissociate milk sugar as well. But, if too much of the above kind of sugar be introduced into the blood, then as a rule defensive ferments do not appear at all.

Further, it is possible that the substances, which we artificially introduce into the body, are not sufficiently fine in structure to produce specific ferments, specially directed against them. The cell gives off its particular disharmonious substances with their specific features stamped on them, while we, on the contrary, bring into the circulation material that has already been altered. This difference may be illustrated in the following manner. Suppose, on the one hand, that two persons start to fight each other with two "specifically" chosen weapons. Such a fight is premeditated; the weapons are precise, and the

defensive measures adopted depend on their nature. In another case two individuals throw themselves brutally one upon the other without any previous choice of weapons; then any means is good, if it only succeeds in overcoming the opponent somehow.

One fact must always be kept in mind. When we introduce proteins or peptones and the like into the blood-vessels, these are never pure compounds. Together with the peptones we certainly introduce into the circulation a number of different stages of the decomposition of proteins. Suppose we take the white of an egg, then without doubt innumerable albuminous substances are present, which greatly differ one from the other. We must not forget the fact, that traces of particular substances are quite sufficient to cause the formation of ferments. The cells, on the contrary, set free substances that are probably of a very precise character.

If we artificially introduce material that is out of harmony with the blood, the animal organism reacts against it with a whole complex of defensive ferments, because our material is always a mixture of substances. It is to a certain degree prepared for all eventualities. It has no actual knowledge of the characteristics of the introduced product. If, at any point, particular cells exhibit a deficiency of function in respect of their metabolism, then there always appears in the blood plasma, from one moment to

another, just a trace of some specifically organized substance, and this is at once deprived of its essential properties by means of the opposing defensive ferment. It might be thought that the same cells which give off the disharmonious material would themselves supply the ferments, and transfer their further reduction into the plasma; but at present there are no proofs for such a suggestion.

This view may be opposed on the ground, that it is difficult to understand how specifically directed ferments can be distinguished in boiled tissues. Many of the finer features in the structure of the substrate must surely be obliterated in the process of boiling. This probably holds only for the physical properties and hardly at all for the chemical. We may boil a substance having a composition A B C D, and another having a structure B C D A, for a long time; both would still retain their original composition or structure, although their physical properties might undergo alteration. Thus, for instance, their rotation might alter, and to this extent their biological behaviour might be affected. Thus there is nothing against the idea, that the substrate against which the ferment is directed may, in spite of its newly acquired properties, be still liable to attack by the ferment. With a key corresponding to a particular lock we can still unlock the latter, even though it has been badly damaged, provided only

that the key can fit into the guide, and push back the bolt. The whole of the rest of the lock may, in this case, have suffered considerable alterations.

The ferments attack a particular substrate at a particular point, and very probably always combine with the groups against which they are directed. It is only secondarily that disturbance occurs of the equilibrium of the compound. So long as this point of attack remains unchanged the ferments are able to act, but the conditions become very different when the much altered product, with all its groupings, is brought into the circulation. If the decomposition is to be a complete one, then a number of ferments has to act. The new conditions, caused by the denaturation, produce their full effect after the introduction into the circulation. When we search for ferments by means of boiled tissues, we expose a variety of proteins to the action of ferments. Only that grouping of atoms, towards which the ferment is directed, has to be considered here. All other groups may be neglected, because it would be scarcely possible to presume that the boiling has produced structural conditions which are accessible to ferments, although the natural substrate was not so accessible. Rather must we reckon with the possibility that a too extensive denaturation will so strongly modify an original grouping, that is accessible to ferments, that the ferment then becomes inactive. The grouping has now become foreign to it.

Much weightier still, at first sight, is the following objection. In the dialysation process we make use of coagulated albumens, when looking for defensive ferments. In the optical method peptones are used which are produced from these. Is there not a contradiction between our methods of research, and the ideas developed above with respect to the cause of the origin of the defensive ferments? If we surmise that the defensive ferments serve the purpose of decomposing disharmonious substances, built up from numerous units, into their single units, then we must further agree that ferments are present which can carry on the decomposition at least so far, that the specific characteristics of the cells are entirely destroyed. Therefore we ought to expect that, where proteins are decomposed by defensive ferments, peptones will also be destroyed provided we take, as substrates, those which correspond with the normal fermentative reduction stages of the original material. If, then, we agree that a substance that is out of harmony with the plasma always has an albuminous character, *i.e.*, that the defensive ferments commence their decomposition at this stage, then there is no difficulty in imagining that the dialysation process and the optical method lead to similar results. In the first case we allow the defensive ferment to commence the decomposition at the albuminous stage, and establish the appearance of the crystalloidal, dif-

fusible stages (peptones). In the second case, we prepare the way for the ferments by producing peptones, which we then allow the ferments to decompose. So we allow serum of pregnancy to act upon a boiled placenta albumen, while in the optical method we use peptone produced from placenta albumen. In the first case, the appearance of diffusible stages of decomposition in the dialysate shows us that the decomposition has begun. In the second case, the change that takes place in the rotation enables us to infer a change in the composition of the added substrate, namely, of the peptone mixture.

Now very often, and probably in the great majority of cases, albumen itself does not pass into the circulation, but rather the products of its decomposition. We may easily understand that the optical method gives us trustworthy results, because it is quite possible that the peptone mixture used may also contain those stages of decomposition which have acted disharmoniously with the plasma. How, then, can we establish a decomposition of albumen? In those cases, for instance, where the cells of the body give off to the blood plasma peptones which still bear the characters of the cell, we start, in the dialysing process, at a higher stage of decomposition than was the case in the blood itself.

Unfortunately, we have no knowledge whatever concerning the nature of the ferments. We realize

them exclusively by the manner in which they act, and that is why, in most questions relating to ferments and their activity, we are only able to answer with conjectures. We may imagine that a ferment is directed against a simpler product of decomposition, and yet that it attacks at the same time a more complicated molecule, in so far as the group through which it attacks the substrate is actually present therein and within reach. It only depends on whether the ferment is able to find a group, corresponding to its own structure and configuration, in the particular molecule. We must, also, not forget that, in a body of high molecular structure, the same grouping may recur many times. In any case we consider it very possible that cases are met with in which the optical method shows a decomposition, while the dialysation process gives a negative result; although it must be admitted that, up to the present, not a single case of this kind has been satisfactorily proved.

All these discussions would be superfluous, if we only knew, on the one hand the nature of the ferments, and on the other the components that are out of harmony with the plasma. As it is, we are simply faced with the fact that ferments, directed against particular substrates, are to be found in the blood serum under very definite conditions. What is entirely new is the clear demonstration of the fact that the animal organism, within certain limits,

generally defends itself, by means of ferments, against compounds that are capable of decomposition and that consist of many elements. New, too, is the idea that by means of these ferments we can judge as to the functions of particular organs. Finally, the idea that the animal organism sets free specifically directed ferments, and that, in so doing, it registers the fact that the components of its various kinds of cells have an exclusive structure corresponding to each kind of cell, is also new.

Objection has been taken to the idea of strictly and specifically directed ferments on the ground, that it is impossible to accept the idea that specific reactions take place, because the so-called "antitryptic power" of Henkel-Rosenthal, the "cobra-poison hæmolysis" of Heynemann, and finally the "catalysator influence" of Weichardt, are not specific. It is forgotten that the fermentative decomposition represents the primary activity, and that those substances, which are in question in the above-mentioned methods, are produced secondarily by the defensive ferments. That, during the process of decomposition, the original characteristic structure of a compound is soon destroyed, we have repeatedly affirmed. All possible stages of decomposition, of the most different origin, can in many respects act identically. Thus, for instance, it is possible to prove conclusively that the hydrolysis of the dipeptide, d-alanyl-glycin, can be

retarded by the addition of optically active a-amino-acids. It is a matter of indifference what kind of a-amino-acid is used, provided only it belongs to the units of the protein. We demonstrate the decomposition of a specifically constructed substrate, while the methods in question are concerned only with the influence of the products that are split off.

Of course, the mere fact, that it has been found possible to found a sero-diagnosis of pregnancy on the principles described above, would not give us the right to speak of a sero-diagnosis of the functions of organs. Further research, based on these principles as well as on the methods described, has, however, given us results which entitle us fairly to presume, that a new road has already been found for the development of our knowledge of the structure and metabolism of cells, under normal and pathological conditions.

As, meanwhile, our knowledge of the physical and chemical properties of the complicated constituents of the cell, and the products of its metabolism, is still very scanty, while in addition the disharmonious components of the plasma only appear in minute quantities, we are not in a position to seek them out by direct means. We have, therefore, to hit upon indirect means, and to find out whether a particular blood serum has ferments at its disposal, which are

able to decompose the substrate peculiar to a particular organ. In a certain sense we give the serum a definite question to answer, when we add all kinds of organs to it, and observe which, or how many, of them are decomposed by it. If we find a decomposition, then we infer a somewhat abnormal activity of the cells of the corresponding organ. We presume that substances have been passed out, primarily from the cells of the organ in question, which have not yet been made sufficiently harmonious with the plasma, and that they still exhibit characteristic features peculiar to the cells in question.

In the future we shall undoubtedly avoid using whole organs and tissues for our researches, but shall select particular types of cells; and we shall have to be particularly careful in deciding, whether the tissue used is normal or modified. It is quite conceivable that, in certain diseases, only those tissues are decomposed which have been modified in a particular manner. In such a case the diseased tissue would be altered in such a way, that the substances that are out of harmony with the plasma would be more or less in disharmony with the cells of the normal organ; by which we mean, that compounds and decomposites would appear, which have completely disharmonious activities. And indeed, it would be possible to imagine that products would be formed that are out of harmony with the entire body,

because the whole organ has become similarly disharmonious.

The fact that the animal organism replies to the invasion of disharmonious substances—which, either taking their origin from the metabolism of certain cells of its organs, or being normal constituents of the cells, pass directly into the blood plasma—by means of specifically directed ferments, is of the greatest importance to physiology as well as to pathology.

Up to the present we have only been able to distinguish three different proteolytic ferments, namely, pepsin, trypsin, and erepsin. In addition to these, we may perhaps reckon as proteolytic the ferments of rennet, and of fibrin. Strictly speaking, erepsin must be excluded, because it is principally directed against the products of decomposition of albumen. Our experience of the defensive ferments induces the supposition that trypsin, for instance, is not uniform in its nature. Of course, it may be possible that there are ferments which, just as a master-key can open various kinds of locks, are able to decompose very different substrates, when these belong to the same type of compound. But it is more likely that, in trypsin, ferments of different kinds are combined, and that in the blood the different components each act separately.

The defensive ferments are, as we have already

pointed out, reagents acting on the characteristic, typical structure of the components of definite kinds of cells. We may illustrate this idea by an example. A great sensation was caused at one time by the observation, that there were unicellular organisms which apparently showed signs of intelligence. It could be seen under the microscope how the unicellular organism, called *Vampyrella spirogyræ*, hurried from one alga thread to another, until it stopped at a particular kind of alga in order to use it as food. However many kinds of alga were offered to it, it would always pick out the same kind. This phenomenon, which seems so amazing at first sight, may doubtless be explained in the following way: Every living being has ferments at its disposal which, as Emil Fischer pointed out, may be compared with keys, and the substrate, against which they are directed, with locks. Just as a particular key generally unlocks and locks only a particular lock, so can particular ferments only decompose or reconstruct substrates of a particular constitution.

The *Vampyrella spirogyræ*, then, hurries from alga to alga, bearing with it ferments, by means of which it intends to convert nutriment into a suitable form. It is always trying to effect an entry by means of its "keys," and it only succeeds in certain cases, namely, when the key fits the lock, which is to say, when the cell-wall of the particular alga is of

such a structure, that the decomposition can be effected by means of the ferments it possesses. A breach is made into the cell-wall; its contents are laid bare, and can then be utilized as nutriment.

This unicellular organism shows us, then, that the various algæ have a very different cell structure. The defensive ferments prove the same thing, and provide us with knowledge which we should be unable at present to arrive at by any other means.

Exhaustive researches are now in progress, which seek to ascertain whether the separate kinds of cells of an organism have the command of specifically directed ferments. We know that every cell requires ferments for the purpose of breaking up the food that is brought to it, or of constructing new compounds out of it. Further, we know that the cell is able to disintegrate parts of its own contents, and to replace them by new material. Is it not probable that specific activities are indicated in this case also? In the course of our experiments in this direction peptones were produced from certain cells, and the decomposition of these peptones by the corresponding cell ferments was then attempted. As a matter of fact, juices obtained from certain organs by extraction or maceration decomposed only peptones or albumen from the corresponding tissues; that is to say, extracted thyroid juice broke down peptones obtained from that organ, but not liver peptones

(E. Abderhalden, A. Fodor, and E. Schiff). The kidneys alone were exceptional, their ferments attacking peptones originating from the most different organs. In all probability this result points to a new function of the kidney, namely, the duty of intercepting all disharmonious substances of a complicated nature, which have been brought to it by the blood, but have escaped the action of the defensive ferments of the blood. The kidney decomposes these, and by so doing renders them useful to the organism. The observations we have quoted suggest the possibility, that the kidneys may be instrumental in supplying defensive ferments to the blood. It would be very interesting to determine the contents of diseased kidneys in respect of ferments, and to find out whether they are still able to perform their duties. By such studies new points of view might be supplied, which would give us a better understanding of the particular diseases affecting this organ. Moreover, exhaustive studies on the specificity of the ferments of cells as such should enable us to prove that each kind of cell has its own structure. We shall also be able, by means of the dialysation process and of the optical method, to make a much better study of cell ferments than has hitherto been the case.

The number of problems, that arise from the facts we have brought forward, is so immense that we shall content ourselves with drawing attention to only a

few of them. In the first place it would be desirable to find out where the defensive ferments arise, and whether they can be met with inside the individual cells themselves. It is, for instance, conceivable that the walls of the intestine, and perhaps also the cells of the liver, are always provided with definite ferments for the purpose of further reducing substances which, though insufficiently decomposed, pass through the gut epithelium; and it is very probable that the leucocytes play an important part in this connection. They circulate rapidly through the whole organism. They are to be looked on as protective organs, which, to use a metaphor, overlook everything with a view to finding out whether order prevails. Some products are eliminated by being absorbed into the body of the leucocytes (phagocytosis); others are attacked by their ferments, and so broken up, and deprived of their characteristic structure. Finally, as mentioned before, the separate organs have to be considered, particularly the kidneys.

But the most important advantage of the methods we have described is, that they will enable us to study the reciprocal dependence of individual organs. Suppose, for instance, we remove the thyroid gland; we then anticipate that another organ, some of whose functions depend on this gland, will have its metabolism interfered with, and will in consequence give

off disharmonious material. The failure of this organ is followed by that of a second, which was accustomed to obtain secretions from the first, and thus we are led to the discovery of wheels within wheels.

Or it may be that investigation of a large material shows that certain dys-functions were attributed, on the basis of our earlier experience, to disease of a particular organ, when all the time it was functioning quite normally. For instance, the following case is quite conceivable. Let us suppose that a very definite function of organ B depends upon organ A. The latter may work quite normally, although B is so modified that it passes into the blood constituents of its own cells. Let us suppose that it is these products against which the secretion, originating from organ A, is directed; then it finds the substances, which it ought to affect within organ B, already in the blood. It combines with these substances, and in consequence never reaches organ B. We then observe the same phenomena as would result if organ A were diseased. The dialysation process and the optical method would, in this case, give the apparently astounding result, that defensive ferments are present in the blood serum which are directed against the components of organ B, whilst those which correspond to the components of organ A would, against all expectation, be entirely absent.

Organ A only appears to refuse to work because, as the result of a primary dys-function of organ B, the secretions are unable to achieve their aim in the proper place. They are caught up too soon.

We must not forget to mention, that it is, perhaps, more often than we imagine, that substances which are out of harmony with the plasma circulate in the blood. We are thinking particularly of disintegration of the form-elements of the blood. That ferments, directed against the components of the red blood corpuscles, can be found in animals that are apparently quite normal, is shown by the fact that, amongst horses and cattle, about 40 per cent. of cases investigated gave a decomposition of albumen which originated from the form-elements (E. Abderhalden and A. Weil).

The following experiments supply a striking indication of the probable cause of this phenomenon. Blood was taken from a rabbit. The serum neither decomposed any organ that was free from blood, nor any organ that contained blood—traces of blood being quite sufficient. Without any further treatment, blood was again taken from the animal after two days, and again the serum gave no decomposition with any organ free from blood. On the other hand, the reaction gave positive results with all organs that contained blood. It was certainly not the albumen of the organs that was decomposed, but the blood

contained in the tissue. The appearance of defensive ferments, after the extraction of blood, is without doubt to be ascribed to the destruction of red blood corpuscles that results therefrom. Is it not possible that the absorption of plugs of fibrin that occlude the walls of the vessels may be connected with defensive ferments, and may not the latter also have a share in the organization of thrombi?

Defensive ferments, which decompose the albumen of blood corpuscles, may be produced by means of injections of hæmolytic blood, and herein lies a source of error which should never be underestimated. Only organs that are absolutely free from blood give conclusive results. Defensive ferments, which decompose the albumen of blood corpuscles, are frequently met with in carcinoma. Any extravasation of blood into the tissues, however slight, will give rise to this kind of defensive ferments.

Within the domain of pathology there is no field which would not lend itself to researches based on the methods we have described. We will call attention to some. In the first place we can try, by means of defensive ferments directed against certain kinds of cells, to discover those organs which are giving off substances that are out of harmony with the blood or the plasma. This would be the case, when a particular organ fails to complete its otherwise normal metabolism. But it is also possible that

decomposites, or secretions, are formed, which are in themselves disharmonious. The future must teach us whether quantitative conditions are decisive or not, but it is at least possible that a secretion of quite normal composition may act disharmoniously with the plasma, when it passes into the blood in too large quantities.

In pathological cases, too, we shall be able, by following up a particular disease, to determine the nature of the reciprocal relations in which different organs stand towards each other. It may be noticed, perhaps, that at the beginning only one organ shows signs of dys-function, that another then follows suit, and so on. We shall also be able to make therapeutical studies. If a therapeutical measure should result in the disappearance of the defensive ferments, the therapy would have to be estimated otherwise than if this were not the case.

A large field of study is presented by all cases of degeneration, such as muscular and nervous degenerations, as well as by processes which result in the formation of decaying products of every kind, such as putrefaction of tissues, or absorption of exudates, of extravasations of blood, or of thrombi, &c.

The infectious diseases obviously supply us with an extensive field of study. On the one hand we shall have to decide whether defensive ferments exist that are directed against specific micro-organisms,

and, further, whether the tissue attacked is decomposed by blood serum. Either the micro-organisms will be able to decompose the tissue—their nutritive medium—in a manner harmonious with themselves and disharmonious with the body, and so produce decomposites out of harmony with the plasma, or else the injured tissue will be altered in such a way as to be no longer able to continue the normal processes of its metabolism. A mass of observations are waiting to be made in this direction.

We may point out that it has been ascertained that, in cases of miliary tuberculosis, defensive ferments exist which are directed against tubercle bacilli. It seems that the serum of cattle suffering from tuberculosis is able to decompose the bovine type only. Caseated lung tissues were not decomposed by the serum of animals which suffered from miliary tuberculosis, but only of those which exhibited caseous pneumonia. These experiments, which were performed with the assistance of Andryewsky, on cattle and cows, are an inducement to further studies.

We may take this opportunity of pointing out, that the dialysation process for the demonstration of defensive ferments offers the great advantage of toxilogically testing the products of decomposition. We may use the dialysate, which must, of course, contain the products of decomposition, either directly, or after complete concentration at a low temperature and

decreased pressure, for all kinds of experiments on animals. It is a pity that hardly any investigations have been made in this direction.

Once we have demonstrated the existence of ferments directed against particular micro-organisms, then the question naturally arises, what influence is attributable to the defensive ferments in a special case. They may act defensively. But it is also possible that it is they which first produce the poisonous substances, when decomposing disharmonious material. The ferment is unable to "know" what will be the result, when it breaks down a particular substrate. It may be that the attacked substrate is quite harmless to the organism, and that injurious substances first appear during decomposition.

If further researches show that the organism defends itself successfully by means of definite ferments, then a road is marked out for therapy to follow. By the direct addition of the necessary micro-organisms, or of certain parts of them, we shall produce defensive ferments which are directed against them, and try to transmit these with the serum. We can determine, exactly, the moment when the defensive ferments appear. A particularly fine basis for experiments seems to be supplied by thromboses, which one might be able to attack effectively by means of properly directed ferments, and so bring about their absorption.

The following observation is important. If albumen is injected into the circulation of an animal for the first time, defensive ferments are found to appear, in the case of intravenous injection, at the end of about one day. If the injection is repeated, say, a month after the defensive ferments have disappeared, then the ferments reappear very much earlier. (Abderhalden and Schiff.) May it not be that immunity partly rests upon the fact that an organism is able to set free its defensive ferments quicker than usual?

Syphilis, also, may certainly be studied from the standpoint we have laid down. We have to consider the affected tissue on the one hand, and the spirochæte on the other, as substrates.

It is also clear that we may suspect the presence, in the serum, of defensive ferments which are able to reduce fats, carbohydrates, nucleo-proteids, &c. The demonstration of proteolytic defensive ferments represents only one special case. We have selected this case because, up to the present, no methods exist for satisfactorily demonstrating lipolytic or amylolytic ferments—in short, those ferments which are directed against the particular constituents we have mentioned —unless one has rather large quantities of serum at one's disposal. We are, meanwhile, engaged in extending our researches to other ferments.

With regard to the infectious diseases, we should

like to discuss more closely what is, in our conception, the relation of the micro-organisms to the cell complex of the host.

Let us return for a moment to the conception we developed at the commencement of this work, according to which the organism, under normal conditions, represents a whole closed in itself. We have already pointed out that the harmony of all the processes going on within the whole complex is perturbed, as soon as disharmonious kinds of cells settle inside it; that is, species of cells which have their own metabolism, and their own specific structure. On the one hand these cells have to be fed, and on the other they give off the products of their metabolism, and perhaps also secretions of various kinds, to the exterior. In order to be able to make use of the nutritive material supplied by the host, which is at first disharmonious with their cells, they also must possess ferments which will make the food accessible. It is conceivable that the substances belonging to the host are first absorbed by the cells, and then transformed in their interior, but it is more likely that the invaders give off ferments externally, which decompose the nutritive media around them, and prepare it in that way for absorption. The resulting decomposites are then taken up by the cell. A reconstruction must be made in any case, and especially when the substances are intended, for the building of new cells.

Experiments which have been carried on with different so-called toxins have shown, without any doubt, that splitting agents are present in them. These experiments, however, do not make it absolutely certain that the micro-organisms secrete ferments, because it is difficult to decide whether the so-called toxins of the trade represent uniform products, and particularly whether they always contain secretions only. As a preliminary condition, then, for the possibility of the existence of micro-organisms amidst a particular complex of cells foreign to them, we require the presence of ferments which enable them to build up the food they require from substances that are in harmony with the cells and the blood of their host. In this case the relations between the configuration of the ferments and that of the substrates are no doubt expressed in their most distinctive form. How often may not a micro-organism penetrate into an organism and die out, only because it is unable to feed on the nutritive medium supplied, while in other cases it is able to settle down because the substrates presented to it can be rendered accessible by its ferments. If all the substances are used up, and none of the same kind are supplied anew by the host at the proper place, then the conditions of its existence are withdrawn from the micro-organism, and it must either perish or find a new "pasture." It may also happen in many cases that

the cells of the host catch the secreted ferments of the micro-organism, or render them inactive in some other way, and in this manner either increase the difficulties of existence for the invaders, or else completely destroy them.

How sensitive individual organisms are, in regard to these nutritive substrates, is shown by numerous laboratory observations on the cultivation of the most varied micro-organisms. We know that many of them only thrive, when very definite substrates are offered to them. The fact, that an alteration of the nutritive medium deprives certain organisms of their means of existence, is shown in the clearest manner by the observation that an infection with trichophyton fungi cures itself at the time of puberty. Evidently the cells of the skin become so modified, at the onset of sexual maturity, that the substrate of the host—the components of the skin—can no longer act as a means of subsistence for the fungus. From this point of view we may well imagine that medicines and other therapeutical means effect a curative action, without directly attacking particular kinds of cells which may be living as parasites in the animal organism, for they need only to destroy the conditions of existence required by the organism in question by means of a modification of its nutritive substrate. It is imaginable that certain means do modify certain cells to such an extent, that their com-

ponents can no longer be considered as supplying nutritive material for the organism that lives in them.

The fact that the cells which are out of harmony with the body are dependent upon nutritive material of the most varied nature for the opportunity of extending their existence, and more particularly for maintaining their species, gives us an insight into the kind of influence exercised by these parasites on the host. In the first place they may act injuriously by the simple removal of nutritive substrates, while, in addition, the preparatory decomposition of the nutritive material may give rise to by-products which are harmful to the organism. We can well imagine that particular cells have ferments at their disposal, which decompose particular substrates in a thoroughly characteristic fashion, and so, for instance, produce stages of decomposition which are quite out of harmony with the cells of the host. The same substrate may be decomposed in the most various ways, right down to its simplest structural units. The idea of an atypic decomposition of substances in harmony with the body, cells, and plasma, by ferments of disharmonious cells, suggests the possibility that micro-organisms, though not actually themselves passing into the circulation substances that are directly poisonous, may act injuriously, simply from the fact that, by means of fermentative decompositions, they form products out of the material

of the host, which seriously affect the metabolism of the latter. It is certainly not necessary in every case that the poisonous substance, the so-called toxin, should originate in the cell of the micro-organism itself; it is just as likely that it is formed, outside the cell, by ferments given off by the latter. When we introduce substances out of harmony with the species or the plasma, we have similarly to reckon with the presence of stages of decomposition, which may be disharmonious with the organism, and capable of producing injurious effects. In this case the disharmonious substrate is the cause of the appearance of a substance, that is disharmonious both in structure and configuration. In cases of invasion by bacteria, we have, on the contrary, a destruction of generally harmonious substances, yet the decomposition is brought about by ferments which are probably of a different kind. The cause of the appearance of disharmonious decomposites is thus to be explained, not by the substrate, but by the nature of the ferments. It is possible that, in time, we may succeed in tracking down in the organism these ferment-like agents that are given off by parasites. For the time being we must be satisfied with being able to point to the possibility of a decomposition of this kind being a cause of injury.

Disharmonious cells may also act injuriously upon the organism, owing to their decomposition inside the

body. The death of a cell of this kind results in the appearance, within the circulation, of substances which are disharmonious. We can make a comparison between a case of this kind and the parenteral injection of substances out of harmony with the body and the plasma; for, in this case, the organism will surely defend itself against the very disharmonious substrate, by depriving the substrate of its specific structure by means of an extensive decomposition. We should then be presented with conditions entirely analogous with the parenteral injection of various substances, or with the invasion, into the circulation, of chorionic cells which are out of harmony with the blood plasma, and the resulting reaction would be entirely similar. But here, too, during decomposition, it may happen that the organism will produce decomposites which are naturally injurious, so that, in any given case, it would mainly depend on whether the intermediate products appeared only in small quantities and were promptly reduced, or whether, conversely, the power of decomposition possessed by the organism is inhibited—either because the decomposites cannot be further reduced or be rejected, or because the ferments necessary for further reduction are not present in sufficient quantity. We can well imagine that the decomposition of the bodies of dead micro-organisms, without the direct participation of the microbes themselves, will give rise to various

disturbances; and we might certainly expect secondary disturbances in the harmonic processes of the whole metabolism of the host, without the micro-organisms as such exerting any direct action.

Finally, we have to consider yet another possibility, that certain micro-organisms produce poisonous substances within themselves, and give them off externally. It is, at present, very problematical, what view we are to take of these substances. Are they substances which play a part in the metabolism of the micro-organisms themselves, or are we dealing with agents which, when passed to the exterior, affect the nutritive medium in some way or another, that is, by either decomposing or reconstituting it? It is quite conceivable that certain micro-organisms have agencies at their disposal, which are able to modify particular nutritive cultures in a particular way. Many pathological observations have proved, that certain micro-organisms require a so-called mixed infection, that is, that certain bacteria alter the cell substance of the host in such a way, that some other kind of bacterium finds conditions favourable for its existence. It seems that this is also the case with some kinds of tumours, such as sarcoma and carcinoma, where a preparation of the medium by certain substances is of great importance. In the future we shall be compelled to appreciate all these possibilities. If we could succeed in limiting more precisely the

conditions under which certain bacteria can exist, mainly on the basis of more exact studies on the composition of the medium, then we should undoubtedly be in a position to employ more objective therapeutic methods. Further, it would be possible to formulate a conception of the injurious activities of certain kinds of bacteria more clearly than we can at present. Unfortunately, it would scarcely be possible to make use of direct methods in this case, unless we were to succeed in cultivating individual micro-organisms on substrates the composition of which we were thoroughly acquainted with. Our progress in the field of the chemistry of the different kinds of cell units, and of their nutritive bases, has brought us nearer to this objective, but a large part of the road lies before us yet, before we shall obtain such an exact knowledge of the composition of certain albuminous substances, such as phosphatides and nucleoproteids, &c., as to be able to properly appreciate differences of configuration, as well as differences of structure. Once we have advanced so far, we shall be able to replace our present conception of "disposition" by definite facts.

The train of thought we have been pursuing is only intended to show that, in considering the question of the injuries which bacteria may inflict on their host, we must not only consider the bacteria as such, but must realize that their entire metabolism is of

pre-eminent importance. It is not the bacteria alone, and the so-called toxins, that have to be considered in the whole question of immunity reactions, but most probably the intermediate products of their metabolism, as well as certain decomposites which are, at any rate partly, formed quite outside the cells in question. And, above all, we have to consider the structure of the organism. The host directs its struggle, not only against the living micro-organism, but also against the particles which appear with the decay of the dead organism, and more particularly against the intermediate products which originate during the preparation of the nutritive medium. The organism attacks every point with its ferments, and tries to decompose or reconstruct anything that is disharmonious with its structure or configuration, or even its physical properties. The more it succeeds in this respect, the more does it deprive the micro-organisms of the conditions required for their existence, and protect its own cells against the injurious action of these substances.

We come, then, to the conclusion, that at least one part of the means of defence, possessed by an organism against infections of any kind, depends on its power of liberating ferments, which attack the disharmonious substances—be they by-products or end-products of metabolism, or products of cellular disintegration—and deprive them as quickly as

possible of those specific properties which are out of harmony with the host. Of course, other processes come into play in this connection. The decomposites are oxidized, reduced, methylated, acetylated, benzolated, &c., and are also, without any doubt, coupled in very different ways with various compounds. The defensive ferments prepare the disharmonious material in a proper manner, so that the individual cells of the body may attack it in their own specific way. The ferments remain unaltered during all these processes. They enter temporarily into combination with the substrate to be altered. Once the decomposition is completed, the ferment is again ready to initiate new reactions—mostly decompositions. An over-production of ferments, in response to an invasion of disharmonious bodies, is therefore unnecessary. The importance we ascribe to these means of defence of the organism against the invasion of disharmonious substances may, however, be objected to on the ground that little is gained by demonstrating the existence of ferments in the blood plasma, and by agreeing that they play an important part in connection with infectious diseases, so long as the ferments themselves are not known by us. We know nothing of their structure, their nature, or their special modes of action; and only become aware of their existence through their activities. The fact, that they are

specifically directed against particular substrates, makes it possible for us to demonstrate their existence, and the knowledge, that ferments play an important part amongst the means of defence of the animal organism against disharmonious substances, indicates this much progress, that we are enabled thereby to follow up, experimentally, phenomena which we meet with under normal conditions in the individual cells of the body. With the help of the ferments the cell is continuously remodelling in a suitable manner the food, which the blood brings to it still in harmony with the plasma, either by way of completing a further decomposition, or of setting up a synthesis. The ferments are the tools of the cells, by means of which fuel is brought into a suitable form, the structure of the cell is completed, and various substances are prepared, which, as secretions, have a more or less definite part to play throughout the organism. If the organism liberates defensive ferments, then its cells effect nothing actually new. A normal process is simply allocated to a specific case. The ferments are adapted to the new kind of substrate, and, if necessary, passed out into the circulation. So that this form of defence against disharmonious substances, which the cell utilizes, may be ranked directly with the normal processes of cellular metabolism—an idea which has always dominated the researches of Paul

Ehrlich. At the same time, a careful analysis of the processes effected by the ferments gives us the chance of determining, with more certainty than has hitherto been the case, what is the nature of the injuries set up by the presence of cells that are out of harmony with the body. Sometimes the parasite takes an active part, sometimes only a passive one, while at other times its influence is extremely varied.

The proof, that ferments play an important part in the means of defence of animal cells against disharmonious substances, opens up new paths for experimental research. It may be long before the true nature of ferments is elucidated; nevertheless, we shall always be getting nearer to the possibility of excluding the second unknown—the substrate. The more we extend our knowledge of the composition and structure of the food material, and of the components of the cells, the more do we find ourselves in a position to make use of substrates of a known structure, which enable us to investigate the ferments in a much surer manner, and to determine exactly how they decompose a particular product. We shall be able to get hold of individual decomposites, and study their properties, so as gradually to penetrate into the mysteries of the effects of infectious diseases, as well as into the principles of immunity.

There is scarcely, in the whole domain of biology, a more stimulating task than that of finding

out how the organism defends itself when disharmonious substances introduce a disturbing element into its metabolism, so delicately poised and harmonized even in its finest details. In these problems the most varied hypotheses regarding cellular metabolism are to be met with. The more the biologist enlarges the limits of his researches, and the more he follows up all general phenomena, the more may he venture to hope that he will gain new means and new lines for the study of special processes. The appearance of the defensive ferments in the animal organism, when it is invaded by substances that are out of harmony either with its body, with its blood, or even with some of its cells, gives us an insight into many problems of pathology, and particularly into those of immunity. Every approximation of fields of thought that are at first sight dissimilar, arising out of observations which allow us to presume common reactions and common processes, must be met with approval. It will then be possible that, by an exchange of results based on very different methods and hypotheses, we shall acquire a wide outlook over the fundamental properties of cells of different origin.

Prolonged observations on a particular case of definite disease will be of the greatest importance. It would be absurd to investigate, say, a hundred

cases of tuberculosis, of paralysis, or of dementia præcox, &c., without carefully considering the clinical aspect of each of them. Above all, one ought to make a continuous study of certain types of disease in their different stages. Thus, for instance, epilepsy ought to be observed before, during and after its onset, at the period of remission, and so on.

The normal individual also offers opportunities for such studies, on such occasions as the advent of puberty, the climacterium, &c.

All the various forms of nephritis supply another important field of research. Does the kidney play an active part in individual cases, or does it only excrete albumen that is out of harmony with the blood plasma —that is, does it, in the main, play only a passive part? The following observation illustrates a case of this kind. Serum, from a female patient suffering from nephritis gravidarum, decomposed placenta-albumen and placenta-peptone with great difficulty. The rotation of the serum was unusually high. When this serum was mixed with that of a normal pregnant patient, a change was observed in the rotation of the mixture; and the fact that this was due to a decomposition was proved by the dialysation method. Neither serum, dialysed by itself, showed any decomposites of albumen. When both sera—the one from the case of nephritis gravidarum, and the other from the normal pregnant person—were subjected

to dialysis together, peptone appeared in the dialysate. This case evidently has to be explained as follows :—

As in every case of pregnancy, the blood was filled with substances out of harmony with the plasma—in this case, obviously, proteins. Normally, these compounds are removed by decomposition, with the help of the ferments. In the case of the female patient suffering from nephritis gravidarum the decomposition was evidently very incomplete, in consequence of which the disharmonious proteins accumulated rapidly, and had eventually to be removed by the kidneys.

This observation agrees very closely with some results obtained by Aschner, who observed that the albumen found in the urine during eclampsia is decomposed by the serum of pregnant individuals, although this is not the case when albumen is taken from a case of ordinary nephritis. It is obvious that traces of a specifically constructed albumen are quite sufficient to produce the effects of a decomposition by specifically directed ferments. Of course, we have no right to conclude, on the strength of this fact, that, because the serum of pregnancy reacts quite specifically towards particular urine-albumens, therefore all the excreted proteins belong to a particular type. The eclampsia may be coupled with an ordinary case of nephritis, or follow it.

Each individual case of albuminuria presents analogous problems. Can the serum decompose urine-albumen, or does it disintegrate the tissues of the kidneys? Is the normal tissue of kidneys decomposed, or only pathologically altered? Let us point out, at this stage, that the determination of the power of rotation of the serum may itself be sufficient to direct us towards various important observations. May there not be a hyper- and a hypoproteinæmia? Is there any albuminuria which is based exclusively on heteroproteinæmia? Bence Jones's albuminuria and the typical albuminuria of pregnancy very likely represent such cases. It would obviously be incorrect to designate such kinds of albuminuria as being conditioned by nephritis.

In this connection we would lay stress on the fact that eclampsia, and the toxæmias of pregnancy, present us with a fruitful field for researches on their special conditions. Up to the present it would appear that the prognosis of eclampsia is the more unfavourable, according as the decomposition of the proteins disharmonious with the plasma is the less complete. It goes without saying that we must not jump to broad conclusions on the strength of these observations. We have never ventured to assert that the deciding factor in eclampsia is the insufficient decomposition of the disharmonious substances; and,

indeed, it is quite likely that this is a secondary phenomenon. The fact, that in cases of eclampsia a disturbance may occur in the functions of the liver, deserves much attention. In two cases—the only two which have been investigated along these lines—a disturbance of the functions of the thyroid gland was also found.

A very useful field of research is also supplied by the tumours. Carcinoma, in particular, should give rise to substances that are out of harmony with the blood plasma, and so to defensive ferments. Our own experience has shown, that the serum of carcinomatous patients decomposes boiled carcinoma tissues, but not placenta. On the other hand, decomposition of carcinoma by the serum of pregnancy was never observed. According to our experience it was possible to obtain an early diagnosis of carcinoma. Moreover, the method may perhaps be of use for controlling the therapeutic methods used in cases of carcinoma, whether they be of an operative or of any other character. From a fortnight to three weeks after the disappearance of the carcinoma cells, the ferments that are directed against cancer should also disappear, if we are to judge from present experience.

Finally, we shall have to consider the diseases of metabolism, and all such phenomena as are etiologically wrapped in obscurity, as, for instance, various dermatoses, sympathetic ophthalmia, &c. Here we

found wanting in this branch of practical application, then it is quite certain that he has not mastered the method, and no special investigations should be undertaken without such a certificate of capacity. No conscientious student should make use of a particular method without having made sure, by some means or another, that he is master of it; *and the diagnosis of pregnancy supplies these means.*

A further important rule is, that we must never allow current theories to mislead us. The assumption of a specific action of the defensive ferments has been established on the strength of the facts derived from experiments, but it has still to be supported by means of exhaustive investigations. Nor should any final conclusion ever be expressed in regard to a particular case, until it has been definitely determined by clinical means. It is in this respect that pregnancy offers such a sure basis. We do not know of any other condition which allows of such a clear and indubitable estimate of method. It either exists or it does not, and is never open to a discussion of "probabilities."

Valuable service is rendered to research as a whole, if every case, which gives an unexpected result in respect of its reaction, is thoroughly investigated. In the first place the dialysation tubes should be changed, or else another organ should be used. If the result always recurs, we still have to exclude the

possibility that, in the organ used, substances are inherent which are also contained in other organs. In saying this we are thinking of such a case as the following. It may be that an individual has some diseased connective tissue in some part of his body. The supporting tissues of the organism also have their metabolism, and are quite able to give off disharmonious products into the blood, especially if they are profoundly altered or broken down. Every organ contains connective tissues, though whether they possess an organically specific structure is highly doubtful. It would certainly be of the greatest importance, if so-called failures were tested with connective tissue pure and simple, and then with the corresponding organ without its connective tissue. It would also be advisable, in the case of unexpected results, to test with the albumens of blood serum, and those of blood corpuscles. Studies of this kind will lead much more quickly to clear results than the hurried collection of data which have probably been all subject to the same source of error.

Amongst other observations which have been communicated up to the present time we shall refer to the following. Epstein found that out of thirty-seven cases of cancerous patients all but one, originating from a cachectic patient, aged 80, decomposed the coagulated albumen of carcinoma, while in no one case was the albumen of placenta

attacked. Further, the serum of forty-seven individuals who undoubtedly had no carcinoma, but who, on the other hand, had at least partly suffered from serious illnesses resulting in general loss of strength, was tested for their action upon the tissues of carcinoma. In forty-six cases no decomposition took place. Ludke and Gambaroff also announce favourable results in regard to the diagnosis of carcinoma.

Some interesting observations of Paltauf may be quoted, as specially advocating the acceptance of ferments which are specifically directed against particular substrates. The tissue of a tumour from a woman, aged 61, was not attacked by the serum of a carcinomatous patient which decomposed carcinomatous tissues, although at the same time the coagulated tissue was decomposed by the serum of pregnancy. The pathological diagnosis was as follows: Malignant chorionic epithelioma.

Bauer draws attention to the demonstration of defensive ferments in the blood serum, in cases of endemic goitre, which are able to decompose the tissues of the thyroid gland. These ferments have also been observed, even when no goitre was present, while the clinical phenomena pointed to a disturbance of the functions of the thyroid gland. We, too, have found a decomposition of the tissues of the thyroid gland in one case of myxœdema. There is no doubt that in these diseases we are dealing, not with an

athyreosis, as was once supposed, but with a dysthyreosis.

The symptoms in Basedow's disease are of particular interest. Here we find decomposition of the thymus gland, of the thyroid gland, and very often of the ovary, as has been established by Lampé, Papazolu, and Fuchs, on the basis of a large amount of material. No other organ was decomposed. It is interesting to note, that normal thyroid gland was decomposed only in very exceptional cases, while Basedow thyroids were always attacked.

This observation points to two possibilities in regard to the production of substances out of harmony with the plasma. Sometimes the normally constructed cell is incapable of completing the otherwise normal decomposition of particular substances, so that materials appear in the blood which still show the characteristic features of the cell, from which they originated. The decomposition is discontinued at a particular stage. A certain analogy with this kind of disturbance of cellular metabolism is presented by those anomalous cases, in which simpler products are not fully reduced. We may refer to cystinuria, alkaptonuria, pentosuria, &c. In the first case cystin, in alkaptonuria, homogentisic acid, and in the last case a pentose, are excreted in the urine. Some forms of glycosuria also belong here. The cells are unable to attack the grape sugar, because no active

ferment is present which can act on this carbohydrate.

Further, the cause of disharmony with the blood plasma may depend upon the fact that cells of a particular kind have degenerated, in consequence of which they have a pathological structure, and give off a disharmonious kind of material, to which the blood plasma is unused.

We may also mention the experiments of Bauer and Reines, made for the purpose of clearing up the etiology of sclerodermy. The actual experiments point to a disturbance of the functions of the thyroid gland; but it is probable that other organs are affected sympathetically. In these experiments, also, it appears that certain organs are decomposed, while others are not.

Fauser, Wegener, Joh. Fischer, Kafka, and others report numerous observations on cases of dementia præcox, paralysis, and melancholia. In the first-named disease defensive ferments appear against the sexual glands and the cortex of the brain. According to our ideas this means that these organs exhibit a disturbance of function, though which organ primarily discontinues its functions cannot at present be ascertained. In cases of melancholia no defensive ferments have as yet been found. In paralysis the cortex of the brain is nearly always decomposed.

These researches naturally do no more than imply

a rapid advance into unknown territory. There are still many stages to go over again, before we can arrive at a definite picture. A serological examination must be first employed, and the clinical diagnosis, which is generally more uncertain, will be compared with it. Often later observations alone will show, whether the serological diagnosis is really correct. It will be particularly necessary to make our clinical description of individual cases as thorough as possible. The serological diagnosis of diseases of the nervous system will attain a great measure of success, when it enables us to isolate diseases, hitherto considered identical, according to the presence of particular defensive ferments, and to find that the clinical course of the disease corresponds with this. In any event, each separate case must be carefully and continuously studied.

Moreover, the above investigations are of special importance for this reason, that they definitely indicate the specific action of the defensive ferments. Fauser and Wegener report that female patients suffering from dementia præcox never decomposed testes, but only ovaries. Conversely, males only attack testicular tissue, but never the ovary. Both authors have also experimented with placenta, and, on the one hand, confirmed the sero-diagnosis of pregnancy, and on the other showed that the male serum never decomposes tissues of placenta. Of

great importance is Wegener's communication, in which he states that, in cases of neuritis, muscle tissue is decomposed.

Finally, we may refer to the fact that experiments have been made with a view to throwing light upon the etiology of diseases of the eye, the causes of which are not yet known with certainty. Sympathetic ophthalmia provides features of particular interest. The researches carried on in this connection, by v. Hippel and Hegener, show distinctly that the defensive ferments exhibit specific activities. The number of observations is as yet too small to enable us to draw definite conclusions therefrom. In connection with these latter observations we may point out, how important it is to follow up every case, clinically, over a long period, and in no case to pay attention only to the disease which gave rise to the investigation. In particular, the further course of the disease should be followed up in all its phases. We have, in the dialysation process and in the optical method, means which allow us to test the functions of organs over long periods of time.

The observations, which we have here briefly sketched out, will certainly undergo rapid extension in various directions. Perhaps it will be found later on, that there are other explanations than those which have been developed here; and it is highly probable that some of the earlier results, which were obtained

by means of other methods, will be brought into line with those acquired by means of the new methods. Thus the discovery, by Hermann Pfeiffer and his pupils, of the existence of toxic compounds in the urine in certain diseases and in certain conditions, should suggest to us that the products, which are formed by the defensive ferments, are also finally excreted. The comparative investigation of the dialysates, however, will have to decide whether there are direct relations of any kind between such products of decomposition and the poisonous components of the urine, and whether we are right in speaking of toxæmias that result from the decomposition of albumens.

Methods in Use.

I.—The Dialysation Process.

The principle of the method: Albumen being a colloid does not diffuse through animal membranes, while on the other hand peptones—the first products of its decomposition—are diffusible. If we put albumen in a dialysing tube and place the latter in water, no albumen appears in the surrounding fluid even after a considerable time. If, however, substances such as pepsin and hydrochloric acid are added to the albumen in the tube, we can soon trace, in the water surrounding the tube, substances which are produced from the decomposition of the albumen. These substances are the so-called peptones and some other simpler products of decomposition. If we desire to test any liquid to ascertain whether it contains any proteolytic—*i.e.*, albumen-decomposing—ferments, we place it in a dialysing tube together with albumen, and note whether peptones appear in the liquid surrounding the dialysing tube. If none are present, we may be sure that the tested liquid

contains none of the active ferments capable of decomposing albumen. Should we detect the presence of peptones, we may be certain that some decomposition of the albumen has taken place. In our special case the fluid to be tested is *blood serum*. It is obvious that the method is exactly the same, when we test, for their capacity of decomposing albumen, such substances as *cerebro-spinal fluid, lymph,* or *extracts from various organs*—e.g., *juices obtained by means of high pressure.*

Dialysing Tubes.—The result of tests for albumen-decomposing ferments by the dialysation process depends in the first place upon the quality of the membrane used. The latter must above all answer two requirements. First of all it must be absolutely impermeable to albumen, and further, evenly permeable to decomposites of albumen. If the tube allows albumen to pass through it, the latter may be mistaken for peptones, unless we apply special tests for albumen. Should dialysing tubes be used which allow peptones to diffuse through at a variable rate, then we should be at a loss in our judgment upon the results of a test, because, as will presently be shown, a control test of the fluid to be tested must always be made, without the presence of albumen, and the results of this test be compared with those of the tests in which albumen has been mixed in the dialysing tube with the fluid under research. Should one

tube be very dense and allow little or no passage at all of the peptones, we should naturally have in this a considerable source of error.

Numerous dialysing membranes are known, of which very few have any real value for our purpose. The dialysation process requires dialysing tubes which can be used over and over again. The best are those supplied by Schleicher and Schüll, of Düren in Rhineland. The tubes of this firm should in no case be used without a thorough preliminary examination, because tubes are nearly always met with which allow albumen to pass through, while others are found through which peptones diffuse with difficulty, so that careful testing of the tubes is indispensable.[13] Further, the tubes must be short ones. No. 579A is a tube specially prepared for our purpose. If tubes be used, which project too much over the surface of the surrounding fluid towards which the dialysing process acts, this gives rise to a very uneven evaporation of the dialysate. The latter soaks into the tube, is carried upwards, and evaporates. Indeed, as we shall see later, everything depends upon the fact that, in comparative experiments, the concentration of the dialysates shall not

[13] Tested tubes are supplied by Schöps, of Halle a/S., but still it is advisable to test them, previous to use, as a matter of security.

be affected by unequal evaporation; and every precaution must be taken to avoid this source of error.

The first duty to be undertaken in making use of the dialysation process, is the testing of the tubes, the so-called standardization of the dialysing tubes. This standardization, as we have already emphasized, implies the *impermeability of the tubes towards albumen, and a perfectly equal permeability for the products of its decomposition.*

(*a*) *Test for Impermeability by Albumen.*—A solution of albumen is prepared. The simplest way is to take the white of a new-laid egg. 5 c.c. of perfectly fresh white of egg are diluted with distilled water in a graduated tube to 100 c.c., and thoroughly mixed by shaking. Of the white of egg, which must be absolutely fresh, only the more fluid portion is used, while all flaky matter or bits of skin—in short, all solid parts—are rejected, as otherwise it is impossible to get a good mixture. Instead of the white of an egg, blood serum may be employed.

Now the tubes to be tested are prepared. They are soaked in cold water for about half an hour. The tubes are then placed in small Erlenmeyer flasks (fig. 7) and 2·5 c.c. of the thoroughly mixed solution of white of egg in water are poured into them. The solution is measured by means of a pipette. While filling the tubes the pipette is placed far down in them, and the greatest precautious must be taken

not to spill any of the egg solution upon the exterior of the dialysing tube. Should this occur, the dialysate would incorrectly show a positive reaction for peptones, when, for instance, the biuret test is applied, since both albumens and peptones give this reaction. To avoid any chance of such an error, the dialysing tube, after having been filled, is closed at the top between the thumb and forefinger and well rinsed in running water. Then the tube is closed in the same manner half way down, and water is allowed

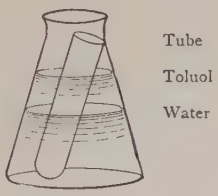


FIG. 7.

to enter the upper part of the tube, so as to wash that part of the dialysing tube which, during the dialysing process, projects out of the dialysate and above the layer of toluol. By moving the thumb and forefinger towards the upper end of the tube we expel the water remaining after washing. All these manipulations have the following object :—

When filling the tube with albumen its interior, near the free edge, may easily come into contact with the pipette. Some of the albumen may adhere to the edge of the tube and dry up in time. At the

conclusion of the test some parts of this albumen may fall into the dialysate and pollute it. During the operation of cleansing the inside of the tubes, care must be taken to prevent water from entering the tubes. Before touching the tubes the hands should be thoroughly cleansed. The use of forceps is much recommended, and these must have wide, parallel, smooth arms.

The rinsed tubes are again put into Erlenmeyer flasks which contain 20 c.c. of sterile distilled water. *The filling of the dialysation tubes must never be done in the same* flasks in which it is intended to carry out the dialysation; something out of the pipette may too easily get into the flask. In order to prevent contamination the surrounding fluid, as well as the contents of the tubes, is covered with a layer of toluol about $\frac{1}{2}$ cm. thick (fig. 7, p. 151). It is best to cover the flasks with watch glasses, unless one is prepared to use stoppered vessels. The dialysation is carried on at the temperature of the room, or, better still, in a closed space at a constant temperature—*i.e.*, in an incubator.

After about sixteen hours—time is of no importance in this test, since the tubes are in this case merely tested for their permeability towards colloids—the dialysation is interrupted. The Erlenmeyer flasks, which should bear corresponding numbers, are placed in a row. By means of a pipette, which is

closed at its upper extremity by the finger and rapidly passed through the layer of toluol, 10 c.c. of the dialysate are taken out, and placed in a test-tube bearing the same number as the corresponding Erlenmeyer flask. This is the best way to avoid mistakes. Of course, for each dialysate a separate and absolutely clean pipette must be used. We do not recommend transferring the dialysates to the test-tubes by means of the same pipette, rapidly cleansed each time after use, because by this means some impurity or other may easily be introduced into the dialysate. Some saliva may very easily enter that part of the pipette which, during the so-called cleansing, remains untouched by the water, alcohol and ether. On the contrary, new saliva is drawn in at each operation if the suction is made by the mouth. Now, when the dialysate is taken up, it is almost certain to be drawn above the level marked upon the pipette, and may then become mixed with the saliva. If test-tubes, graduated to 10 c.c., are to hand, then these tubes may be employed in the following manner: After removing the dialysing tubes, the toluol is drawn off and the dialysate is poured directly into the test-tube. It is of no great importance, in the biuret reaction, to consider quantities to the minutest exactness, nor does a little toluol do any harm.

Now, to each test-tube is added about 2·5 c.c. of a 33 per cent. caustic soda solution. The whole is shaken

sideways to and fro. The mouth of the tube should not be closed with the finger, as in this manner some impurities may easily enter the mixture. Very often the dialysates become turbid upon the addition of the caustic soda solution, but this does not interfere with the reaction. In order to test for diffused albumen we have different methods at our disposal, of which the biuret reaction has been found to be the best. One could also make use of the precipitin formation that appears when prepared serum is employed, but such serum is not always at hand. Further, we may use ninhydrin, but it is not so sensitive to albumen.

Ninhydrin reacts, amongst others, with compounds which carry an amino group in *a* position to the carboxyl group; when it produces a bluish-violet colour, if the concentration of the reacting compounds is sufficiently strong. The albumen molecule contains a few free amino and carboxyl groups, and as soon as it is decomposed, these groups are set free. The ninhydrin reaction becomes stronger the more the albumen is decomposed, provided the various stages of decomposition are not withdrawn. At each stage an amino and carboxyl group are set free. The biuret reaction manifests itself quite differently. The greater the fractional decomposition of the albumen, the weaker is the biuret reaction. As soon as we pass a certain limit of decomposition the reaction ceases.

The biuret reaction is unfortunately rather difficult to detect when it is a case of demonstrating slight traces of the reddish-violet coloration. This is due to the fact that the eye is but slightly sensitive to these tints. Again there are great individual differences. If the observer is unable to detect a light biuret reaction then he has to rely on standardized tubes; or else he must make use of the ninhydrin reaction and try, by means of lengthy dialysation, to increase the quantity of albumen in the dialysate, so far as the tubes are permeable to albumen. Seeing that white of egg, as well as serum, always contains substances which diffuse and react with ninhydrin, we are bound to find out, by means of a standardized tube, what quantity of a given albumen solution we may use without running the risk of the dialysate showing a ninhydrin reaction. How to perform the ninhydrin test we shall describe later, when we give the test for equal permeability to decomposites of albumen.

The biuret reaction is performed as follows: To the mixture of the dialysate with caustic soda about 1 c.c. of a very much diluted copper sulphate solution —*e.g.*, 1 in 500 c.c.—is added. This solution is run down by means of a pipette along the inside of the test-tube, so as to obtain a surface layer. Then we observe by transmitted light the dividing line between the blue layer, which often, however, appears

turbid owing to the deposition of copper hydroxide, and the quite colourless liquid below. The slightest trace of a pinkish-violet colour is a proof that the tube from which the dialysate was procured is unsuitable. Often the presence of albumen is shown by the fact that the precipitated copper oxide dissolves after a time—in about half an hour—and a clear violet layer appears which gradually diffuses into the other liquid. With this test it is better to be over-cautious, and the tubes should be rejected each time the biuret reaction gives doubtful results.

(*b*) *Testing of the Dialysing Tubes for equal Permeability to Decomposites of Albumen.*—Tubes, which do not allow the passage of albumen, must first of all be thoroughly cleansed. Their contents are poured out, and they are then placed on a sieve and rinsed for about half an hour in clean running water.

For the sake of security they are put in boiling water for not more than half a minute. We may also point out that experience has shown that boiling the tubes is not very good for them, for they easily become too dense. After this, 2·5 c.c. of a 1 per cent. solution of silk-peptone are poured into them; the tubes are again carefully rinsed in cold water, one by one, and are then placed in Erlenmeyer flasks filled with 20 c.c. of sterilized distilled water (compare pp. 150-152). The latter is covered with toluol. In

this case also the dialysis is carried on in an incubator, in order to expose all the tubes to approximately equal conditions.

After some sixteen hours the ninhydrin reaction is applied. As this reaction depends so much upon the degree of concentration, it is advisable to carefully guard against the following sources of error. First of all, the dialysate must not be allowed to evaporate unevenly. To avoid this, an excess of toluol is added, and the Erlenmeyer tube is preferably covered with a watch glass. It is clear that, should the different dialysates evaporate unevenly, the ninhydrin reactions would be of varying intensity. The second source of error lies in the boiling of the separate test-tubes, which is applied in order to produce the formation of the colouring substances. We shall return to this presently.

In the application of the ninhydrin reaction we must never forget the fact, that ninhydrin is a most delicate reacting agent for albuminous substances, peptones, polypeptides, and amino-acids. Perspiration reacts very readily with ninhydrin, as do also the epidermic scales, &c. It is most important to avoid any contact of the dialysing tube with the hand; only sterilized forceps should be employed for holding them, and all the apparatus in use should be absolutely clean and dry. One must never rely upon any rapid drying methods. In the first place, it will not do to

transfer the dialysates into the test-tubes by means of one pipette. It is essential to have at one's disposal for the actual tests as many different pipettes, graduated to 10 c.c., as there are dialysates to be handled. The test-tubes must also be absolutely clean and dry, and they must be of exactly the same width. Pouring the dialysates into the test-tubes is not admissible, because the toluol may easily spoil the reaction, chiefly by preventing satisfactory boiling.

In detail one proceeds as follows: As before, the pipette, closed at the top with the finger, is passed through the toluol layer, and 10 c.c. of the dialysate are withdrawn. The pipette is kept closed when passing through the toluol layer, in order to prevent any toluol from entering it. After transferring 10 c.c. of all the dialysates into the test-tubes, using separate pipettes for each, we add to each test exactly 0·2 c.c. of an accurately prepared 1 per cent. solution of ninhydrin.

For accurate measurements a capillary pipette of 1 c.c. is used. The ninhydrin solution is prepared as follows: ninhydrin is usually sold in 0·1-gr. packets, and this quantity is shaken out of the tube into a measuring flask marked to 10 c.c. The tube is best emptied by tapping it against the inside of the mouth of the measure, though it is not possible by this means to transfer the whole of the 0·1 gr. of ninhydrin into the measure. The rest of the nin-

hydrin must be dissolved with distilled and sterilized water; this solution is poured into the measure, and the tube is again rinsed several times, after which the measure is filled up nearly to the mark. Ninhydrin dissolves sparingly in water, and in order to dissolve it quickly it must be heated a little. For this purpose it is best to stand the measure in the incubator. As soon as the solution is effected, the contents are cooled, and filled up to the mark on the flask.

The ninhydrin solution is not absolutely stable. It is liable to infection, and is also sensitive to the action of light. It may be kept in a brown flask, but this is not necessary so long as one prepares only 10 c.c. at a time, a quantity which is quickly used up.

After all the test-tubes containing 10 c.c. of the dialysate have been filled with 0·2 c.c. of the ninhydrin solution, a boiling-stick is placed in each. The latter is absolutely essential, because only a very even ebullition will produce a properly comparable colour reaction. The boiling-sticks used in the trade are divided into segments of about 10 cm. long; these must be boiled in distilled water, dried at 60° to 70° C., and kept in a tightly closed glass vessel. Boiling-sticks must not be stored in a damp condition; for on the one hand their use in this state may give rise to error, owing to the uneven amount of

water present, while on the other hand mould may easily appear. Again, the boiling-sticks should never be dried at too high a temperature, otherwise they may turn brown, and in that case they give off a brown colouring matter during boiling, and thus render an exact reading impossible. They must never be touched with the hands, but should always be placed in the test-tubes by means of forceps.

The process of boiling is now started, and the manner in which this is carried out is of the greatest importance. Boiling must be intensive; at the same time every precaution must be taken to avoid the slightest spilling, as also to prevent uneven evaporation. When all the liquids to be tested have been boiled, we must assure ourselves that they are at the same level in all the test-tubes. It is best to use large test-tubes upon which the volume of 10 c.c. is conspicuously marked. It is then easy to ascertain whether the very important point of even boiling has been accurately carried out.

The test-tube is first held by means of a holder in the centre of a Bunsen burner, the flame of which must be a full one. One then watches carefully for the moment when the first bubbles of gas appear on the sides of the test-tube, which only takes a few seconds, and calculating from this moment one boils for exactly one minute. After ten to fifteen seconds a vivid ebullition is observed, and as soon as this point

is reached the test-tube is brought to the edge of the flame, and the boiling is continued at the middle height of the flame (see fig. 8). In this way it is possible to carry out the boiling continuously and energetically, so that the liquid travels over more than half of the test-tube, without any danger of over-boiling. Not for a single moment should the attention be allowed to wander from this process, for

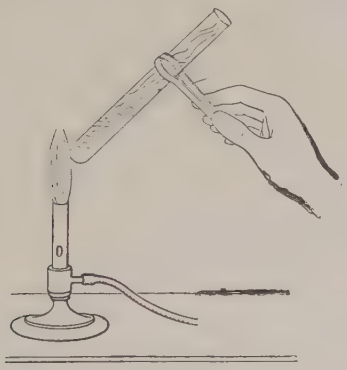


FIG. 8.

(*a*) Test-tube holder; (*b*) boiling-stick.

everything depends upon the accuracy of the operation. If the ebullition is too weak, then under certain circumstances the reaction may fail altogether, while, if the rate of ebullition differs in the different tests, we get a difference in the intensities of the colorations. The results, in short, are inaccurate.

After the lapse of half an hour a comparison of the

intensity of the blue coloration is made in each case. It is soon found that a particular intensity prevails. All the tests which show a greater or smaller intensity than this are carefully noted, and the tubes from which the dialysates in question were obtained are rejected. In this case, too, it is necessary to be very accurate, otherwise the actual experiments may easily lead to deceptive results. Thus it may happen that serum alone, and serum added to a given organ, contain diffusible compounds which react with ninhydrin with precisely equal difficulty; yet on testing the dialysate in the experiment serum + organ, we may get a positive reaction, because the tube was more permeable to decomposites of albumen than the tube used as a control.

The tubes that are equally permeable in this respect are now carefully rinsed, then plunged into boiling water for thirty seconds, and finally brought into a sterilized flask. Sterilized water is added, together with an equal quantity of toluol, the fluid being calculated to fill the flask exactly. The tubes are now ready for use. They are taken out of the flask with sterilized forceps and must, if possible, not come into any contact with the fingers during all the manipulations.

Preparation of the Substrates (Organs).—As material for these tests we use either an albuminous body or else a mixture of these bodies—*e.g.*, an organ. The

manner in which a substrate is prepared is of the greatest importance for the whole success of the dialysation process, and, unless one adheres to the directions in every particular, one is bound to meet with unsuccessful results. These, however, can be successfully avoided if the preparation of the substrate be carried out with proper attention. The principle of the matter is, to obtain substrates which contain coagulated albumen and are absolutely free from diffusible substances which react with ninhydrin. We shall demonstrate the method of obtaining the substrates by means of the preparation of coagulated placenta. Other organs are treated in exactly the same way; only, those which are rich in fats and lipoids have to be previously extracted with carbon tetrachloride in a Soxhlet apparatus. The same applies also to tubercle bacilli. Placenta can always be procured in a fresh state, whereas in other cases we have to deal with organs from dead bodies. In the latter case the dissection should be made at the earliest opportunity. The best corpses are those of accidents. If prolonged agony has been undergone previous to death, the organs are almost useless. It is very important to test the organs for pathological changes; and it is absolutely essential to state in what condition the organ used was found, for different results could easily be obtained if one observed used normal organs while another

made use of abnormal ones. The question whether the organs of animals may be used will be discussed later.[14]

The organ must be absolutely freed from blood, a condition that can be attained in the case of different organs with varying facility. Placenta and lungs, for instance, can be easily washed so as to free them from blood, or the blood may be rinsed out through the large blood-vessels; whilst the liver, kidneys, and particularly the uvea, are freed from blood with great difficulty. With the latter there is scarcely any other means of proving its suitability, than by experimenting comparatively with serum from individuals with healthy and diseased uvea respectively. The pigment prevents us from discovering the last traces of blood.

The fresh and still warm placenta is first freed from blood clots by mechanical means, the membranes and the umbilical cord being removed at the same time. Then the placenta is cut into small pieces, about one inch square or less, and these are crushed in a current of water, for which purpose they are best placed on a sieve. Water is allowed to run continuously upon the pieces of placenta, each piece being pressed between the fingers. From time to time the pieces are placed in a cloth and squeezed in it. The washing of the placenta must never be

[14] See also p. 27.

interrupted. Pieces to which coagulated blood adheres, which cannot easily be removed, are rejected. Finally, they are placed in a mortar and broken up with a pestle, by which process the last traces of blood are eliminated; and then the connective tissue can be removed. We now have a snow-white tissue, which is immediately boiled. The whole process takes from one to at most three hours, according to the kind of tissue employed.

The extraction of blood can also be effected by thoroughly washing out the organ through the blood-vessels; but in this case, the organ must be washed out again after being broken up. If the extraction presents any difficulties, one can often attain one's object by covering the tissue in the fresh state with a very thick layer of common salt. The mixture is allowed to stand for two to six hours in an ice-chest; the salt is then dissolved, and the washing carried on in the usual manner. One must never preserve an organ from which the blood has been incompletely abstracted, in any particular manner, with the intention of completing the process later on. All preservation media produce coagulation and alteration of the blood. The smallest blood-vessels always contain, in that case, small quantities of blood constituents. We must also give particular warning against the use of bleaching agents, as, for instance, hydrogen peroxide. The

red colour of the blood indicates to us that it is still present. If we use hydrogen peroxide, then we lose any control over the blood contained in the tissue. If one is not quite certain of the fact that the organ is free from blood, one should squeeze out a few pieces of it in a little water, and examine the fluid with the spectroscope.

About a hundred times more distilled water than there is of the tissue is placed in an enamelled vessel and then brought to the boil. The tissue, having been absolutely freed from blood, is placed in the boiling water, for every litre of which it is advisable to add about five drops of glacial acetic acid. This is boiled for ten minutes, and the boiling water is passed through a sieve; the tissue is thoroughly rinsed for about five minutes with distilled water, and the same process of boiling is repeated, using fresh water without the addition of acetic acid. The boiling, the pouring off of the boiled water, the rinsing of the tissues, and the renewed boiling are repeated about six times without interruption. If it is necessary to cease boiling, then one must never forget to pour a fairly large quantity of toluol on the top of the boiled water containing the tissue. If this be omitted the tissue is liable to become infected, and then some hours of boiling may be necessary in order to free the organ again from extractive substances which react with ninhydrin.

If a centrifuge be at one's disposal, the boiling water is centrifuged at a suitable speed. This is still more necessary when one is working with finely minced organs or bacteriological cultures and the like, otherwise too much of the material would be lost when pouring off the water.

After the sixth boiling only five times the amount of water at most is used. The smaller the amount of water employed, the more exact is the result of the test for the extractive substances that react with ninhydrin. In every case as much water must be present as will be needed to continue active boiling for five minutes without the risk of burning, the smallest possible vessels being used. Then a certain quantity of boiled water is filtered through a hardened filter paper. To 5 c.c. of the filtrate is added at least 1 c.c. of a 1 per cent. aqueous solution of ninhydrin, and the mixture is boiled (as described on p. 160 seq.) for one minute. If, after half an hour, not the slightest trace of a violet coloration manifests itself, the organ may be considered as suitable, provided it still remains snow-white. Only the tissues of the liver, the spleen, and the kidneys do not appear quite white. Should the tissue turn grey, or even brown, during boiling, this is a proof that it was not absolutely freed from blood, or that the boiling was not conducted properly. Should the particular test prove positive, the boiling must be continued—*i.e.*, the water must be poured

off, the organ be thoroughly rinsed in distilled water, and boiled over again for five minutes with not more than five times its own quantity of water. It is filtered again through a hardened filter; to 5 c.c. of the filtrate is added at least 1 c.c. of ninhydrin solution, and the mixture is boiled for one minute.

Before the organ is put by for keeping, it is spread upon a white glass plate or a sheet of white paper, and every separate piece is thoroughly examined. Should brown spots or other doubtful points, which cause one to suspect the presence of coagulated blood, be noticed, the pieces affected must be thrown away. Only by conscientiously and carefully adhering to these rules can results be expected which are free from all objection. An organ, which has given a whole series of correct results, may lead us astray if even one single piece containing blood happens to be used.

As soon as the organ has been tested in the above manner for the absence of any piece that may contain blood, and as being free from extractives which react with ninhydrin, it is immediately placed in a bottle, with a well-ground stopper; the bottle having been previously sterilized. Then a little sterilized distilled water and a good deal of chloroform and toluol are added, the bottle being filled in such a way that the stopper comes into contact with the liquid. A thoroughly well-prepared organ should

preserve indefinitely, and it only becomes useless again by being contaminated. There are various contingencies that may spoil a perfect organ. In the first place, it must be taken out of the bottle only by means of sterilized forceps. None of the sample taken should be put back into the bottle if it has been exposed to the risk of infection, or been left lying about, and so on. The bottle must be kept filled with toluol, otherwise part of the tissue may adhere to the neck of the bottle. If such a piece protrudes from the level of the toluol it decays, and finally drops down on to the rest of the tissue. The bottle containing the organ should be kept in an ice cupboard.

Bacteria and other living organisms may be prepared exactly in the same way as tissues. Boiling is also resorted to in these cases; and the same rules hold good. It is obvious that organs can be separated into their tissues. The more special the problems to be dealt with, the more does one limit oneself to a very definite tissue.

All organs which are very dense in structure, and which become hard when boiled, require special treatment. Carcinomas, myomas, &c., may appear snow-white and still contain blood, so that in these cases the pieces have to be cut into very minute particles in order to prevent mistakes.

Every organ must be standardized. Placenta is only useful so long as it is not decomposed by the

serum of carcinomatous subjects, or of individuals with salpingitis, tuberculosis, and the like. Carcinoma is correctly prepared if it is not attacked by the serum of pregnant individuals.

Above all, the organ should be tested by means of cases which contain ferments acting against the components of the red blood corpuscles. Cases of blood effusion are excellent testing agents for the absence of blood in the prepared organ. Or disharmonious blood—in this case human blood—is injected into an animal, and its serum is tested against coagulated red blood corpuscles and the organ to be employed.

In conducting these experiments we must be able, with absolute certainty, to prevent the decomposition of all proteins other than those belonging to the actual organ itself. It is clear that serum, which contains a defensive ferment against the components of the form-elements of the blood, will decompose every organ containing blood—that is, it will split up, not the proteins of the organ, but the components of the blood within the organ. The importance of a clear recognition of this circumstance may be gathered from the fact that serum of normal horses and cattle decomposed red blood corpuscles in about 40 per cent. of cases. Further, it was found that serum taken from animals that exhibited hæmatoma produced decomposition with every kind of organ containing

blood, whilst organs freed from blood and subjected to parallel tests were left unattacked. This fundamental rule, of completely freeing the organ in question from its blood, is often transgressed. If the serum does not contain any defensive ferments against the form-elements of the blood, then, of course, even an organ containing blood may give correct results. As, however, mistaken results are liable to occur, such an organ should, as a rule, never be used.

It is advisable never to use one particular organ exclusively for testing a definite problem; and one should always work with controls. For instance, placenta is always tested with serum from obviously non-pregnant persons. Male serum should also be employed. Should cases of diabetes, for instance, be tested exclusively with faulty preparations of pancreatic gland, then in most cases a "decomposition" would be found. Such mistakes are avoided by using thoroughly prepared organs on the one hand, and by means of control experiments on the other.

It is of fundamental importance to establish the morphological state of the organ used, and its origin. It is possible that, in a given disease, a normal organ is not decomposed, although the same organ is readily attacked, if it has already undergone particular pathological changes. Thus it is quite possible that, for instance, a normal thyroid gland would not be decomposed by Basedow serum, while a gland

originating from a morbus Basedowi would be subject to decomposition. Just as every case examined has to be thoroughly tested by clinical means, and its further course closely followed up, so also must the substrate to be employed be characterized exactly. A bare statistical compilation of cases, with percentage accounts of faulty diagnoses is unworthy of scientific publication. Each separate case must be clinically investigated. This is the reason why the fruits of these researches are bound to fall into the hands of clinical observers. The physiologist can only note one case after another without being able to characterize them individually, or even to observe them continuously, and, in consequence, we can expect little help from his side.

A very important question is whether, instead of human organs, the corresponding organs of animals may be used in experiments with human serum.[15] It would naturally be a great advantage in all these researches if this were the case. Our earliest researches enabled us to state the fact that human placenta can be replaced by that of animals, and vice versa.[16] We have made further experiments with the brain and other organs, and have obtained good results. It seems that organs which have the same

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[15] Compare here also p. 27.

[16] Compare also the works of Schlimpert and Issel (Lit. 74), of v. Hippel (Lit. 119), Fuchs (Lit. 111).

function to fulfil, in the animal kingdom have common properties in their structure. In spite of our favourable experiences we have not ventured generally to recommend the use of animal organs. It is still very difficult to find a proper balance amongst the contrary results of many observers, and were we to change the type of substrate without sufficient experience, we should arrive at still more divergent results. This is the reason why it is particularly necessary to use organs of the same species as that to which the serum under investigation belongs, as well as those of a different type. Only when it is established that harmonious results are obtained ought we to be satisfied with non-specific organs, and always under the condition that no substrate is used which shows definite pathological alterations.

Means of Obtaining Blood Serum.—Three conditions have to be complied with. The serum must be as poor as possible in diffusible substances which react with ninhydrin, and this is attained by taking the blood in a fasting condition. In all cases in which the albuminous metabolism is very rapid, in cases of disease which are accompanied by decay of the tissues, as in the case of carcinoma, in cases of absorption of exudates and transudates, in all purulent processes, and lastly, in effusions of blood, the blood always contains a larger quantity of such compounds. The blood serum must further be absolutely free from

hæmoglobin, and in doubtful cases the spectroscope should be used.

The serum must be completely freed from its form-elements, a point which is often neglected. A serum may appear absolutely clear, and yet contain millions of red blood corpuscles. The serum must be treated with a good electric centrifuge until the tube shows no trace of blood corpuscles, either on its sides or at the bottom. The serum, after each treatment with the centrifuge, is drawn off with a pipette and transferred to another tube, and during this operation, in order to avoid any contact of the pipette with the red blood corpuscles, the tube is placed upon a mirror. One can then see exactly where the end of the pipette is at any moment. The blood is best taken with an absolutely dry needle and placed directly into a sterilized centrifuge tube, or, better still, into a small Erlenmeyer flask. The blood is allowed to clot spontaneously, and is watched until the serum separates out. Any mode of procedure which accelerates the separation of the serum increases the risk of hæmolysis. The blood should not be placed either in an ice-chest or in an incubator, but should be left simply at room temperature. In the first case, the risk of hæmolysis is very great; in the second, autolysis of the form-elements generally results. Serum is generally obtained in a considerable quantity after five or six hours, but if enough has not

separated out one makes use of the centrifuge. In the first case the serum is poured into a centrifuge tube, and centrifuged for about five to ten minutes. It is then easy to ascertain that the serum, which was apparently free from solid elements, has now given off a whole layer of red blood corpuscles during the process of centrifuging a second time. Should this remain in the serum, then during the dialysis hæmolysis would take place in the dialysing tube, and the experiment would give faulty results.

It happens, usually, that the experiment is so arranged that, say, 1·5 c.c. of serum are taken from the centrifuge tube and employed as a control. Only after this do we remove more for the test, organ + serum. If at this point the directions are not followed exactly, it may easily happen that red corpuscles are found in the test, organ + serum. Hæmolysis appears during dialysis, and then we have exactly the same conditions as arise when organs are used which contain blood; only in this case the contents of the corpuscles are found, not in the tissue, but in the serum. It is from non-observance of the rules given that we get the observation that a serum, which is absolutely free from hæmoglobin, appears quite red at the end of the experiment. It is the diffusion of water from the outer fluid into the tube that has led to the hæmolysis of the red corpuscles which, though present, have been overlooked.

It is sufficient to use 15 to 20 c.c. of blood. For sending away, only serum should be used which has been centrifuged completely. The latter must in any case be centrifuged again. The serum should not be more than twelve hours old, even though it has been collected and preserved in a really sterile way. The taking of the blood, its collection, and its manipulation must be done aseptically.

Performance of the Experiment.

In carrying out a dialysation test the following fundamental rules must be obeyed, of which not one is unimportant :—

(1) Extreme cleanliness is the first condition for ensuring success in the experiment. This applies to the surroundings, and to all the utensils employed. Pipettes, test-tubes, Erlenmeyer flasks, &c., must be thoroughly cleansed and absolutely dry.

(2) The water used must be thoroughly sterilized distilled water. So-called distilled water often proves to contain numerous germs of every description. If one uses water like this as the outer fluid in dialysis, then the way is laid open for all kinds of mistakes.

(3) The work is performed, as far as possible, aseptically and antiseptically.

(4) In the room in which the experiments are in progress neither bacteriological nor chemical work should be allowed. Above all, an incubator must be

specially reserved for these experiments, nor is it possible to allow the incubator to be used at the same time for bacteriological purposes.

(5) Before starting it must be ascertained that all utensils are to hand and in perfect condition.

(6) Experiments can only be carried on with good light. It is impossible to carry on more than five or six experiments at the same time with the necessary care.

(7) Before successful tests can be expected, one must not only be certain of a perfect knowledge of all the details of the method, but a thorough knowledge of their fundamental principles is most essential. It is not sufficient to know the method thoroughly, one must have a perfect command of it, and, as it were, live in it. No one is able to stain tissues perfectly for the first time, even though he be guided by the strictest directions. Even simple chemical methods require practice, and the most elementary analyses sometimes fail. Even the Kjeldahl method, which is so easily handled, requires to be thoroughly learnt. Should a failure result, no one would think of communicating it while blaming the method; he would never rest until the cause of the error was found. The statement, "We have been working in the strictest manner according to the given directions," I treat with scepticism on the basis of a rich experience. Such great offences are often

committed against the fundamental rules of the whole method, that errors are bound to occur. Therefore, we must not ignore a method because it requires careful working. It is quite possible that with time we shall arrive at more simplicity in our manipulations, and technique may place some further means at our disposal. But it is as yet too early to try to introduce modifications in the manner of working of the two methods, after a whole number of observers have obtained good results by their means in their present form. The principal requirement of any method is, that we should not rest until the cause of error is found in each case that occurs. This is the only way of avoiding them.

First of all blood is taken. If there be any doubt regarding the suitability of the substrate it is advisable first to test the organ, so as to avoid withdrawing the blood unnecessarily. This test should be repeated immediately before performing the experiment. The blood is allowed to coagulate spontaneously at the temperature of the room.

Immediately before beginning each experiment the organ is tested, and this important rule must never be neglected. It may so happen that all the parts of an organ have been freed from all extractive substances reacting with ninhydrin, except a piece here or there. It should be the duty of the observer to note down each time in his record : " Organ tested."

So much of the tissues as are necessary for the experiments to be performed are taken, and to them is added at most five times their quantity of water. If any difficulty arises in the boiling, which may be traced to the insufficient quantity of tissue used, then more tissue is added, the excess of the organ being immediately put back into the bottle that contains the rest, in case it may be wanted later on. If the organ is left lying about for any time it becomes infected. An organ should never be boiled without being previously tested. It should not show any places that contain blood.

Further, the tissue must be shredded into small particles before it is boiled. It would be a great mistake to boil the tissues in large pieces and to use them later in the form of little pieces, for it might often happen that inside the big pieces products were enclosed which diffuse and react with ninhydrin, and they would not be noticed because they have not reached the outside. If, for instance, a lentil is boiled as a whole, the water does not readily show any ninhydrin reaction, but as soon as the lentil is broken up and boiled an intense reaction is observed. In the process of boiling the outer part coagulates, and thus tightly encloses the inner contents. Exactly the same thing may happen with other tissues. Therefore, before the experiment, the organ must be boiled in the same way as it is intended to be used, *i.e.*, in a shredded form.

The best way is to carry out the boiling in a test-tube for five minutes. It must be boiled energetically, and then filtered through a small hardened filter; after which, at least 1 c.c. of the 1 per cent. ninhydrin solution is added to 5 c.c. of the filtrate. Should one have less than 5 c.c. of the filtrate there is no harm in boiling with 1 c.c. of ninhydrin, because the stricter the conditions of these tests the better.

Boiling is performed (as described on p. 161) for one minute with the aid of a boiling rod. Only in cases, where the solution gives no traces whatsoever of a violet coloration, can the organ be used, and one must wait half an hour before one can establish its presence or absence. Should the organ not be required for immediate use, it must at once be covered with a layer of toluol. Should this test still give a coloration, then the substrate must be boiled over again with five times as much distilled water, until the test shows negative results.

Now, as many standardized dialysing tubes as are required are placed into empty, dry Erlenmeyer flasks, and about $\frac{1}{2}$ grm. of the organ is poured into the tubes. This quantity is previously placed upon a piece of blotting paper, and dried by squeezing it strongly. Were the organ placed in a wet state directly into the tubes, a reaction which would give a weakly positive result might turn out negative,

owing to the dilution of serum so caused. The tissue should never be handled with the fingers.

To the tubes containing the tissue 1 to 1·5 c.c. of serum are now added. A rule should always be made of arranging this experiment first. Afterwards from 1 to 1·5 c.c. of serum are placed in an empty tube (control test). Then the tubes are thoroughly rinsed with distilled water (as described on p. 151), and placed in Erlenmeyer flasks which have previously been filled with about 20 c.c. of sterilized water. Then a large amount of toluol is poured into the tubes and over the liquid outside, care being taken that the part of the tube which projects from the liquid should be soaked with toluol. At this stage of the experiment the following sources of error may arise. First of all, water may get into the tubes while they are being rinsed. If the work is not carried on in a scrupulously accurate manner considerable dilutions may occur. The tube must be completely closed during this operation. I have lately been in a position to observe a second source of error which may arise. Contrary to instructions the flask was filled with 20 c.c. of water and a large quantity of toluol, and only then was the tube and its contents immersed. In this case the liquid in the flask was raised to such a level that it passed from the outside to the inside of the tubes. Besides, the tube came into contact with the neck of the Erlenmeyer flask

in many places, and here part of the liquid became enclosed by capillary action, thus forming a kind of communication between the contents of the tube and the liquid outside. From these observations it follows, that the toluol should never be introduced before the dialysing tube has previously been immersed in the 20 c.c. of water, in which case the quantity of toluol added can be accurately controlled, and care can be taken that both the inner and outer surfaces of the tubes shall project at least 0·5 c.c. over the toluol layer. Moreover, only wide-mouthed Erlenmeyer flasks should be used.

Then the flasks are placed in an incubator at a temperature of 37° C. At a higher temperature the ferments would be destroyed, and at a lower temperature the decomposition would be too slow.

After about sixteen hours the experiment is stopped. A thick layer of toluol must still be found upon the contents of the tubes, as well as on the surrounding liquid, at the end of the experiment. The Erlenmeyer flasks, carefully numbered, are best arranged in no special order. Then the tubes are taken out of the flasks, and placed, right up to the end of the experiment, into empty Erlenmeyer flasks. In withdrawing the tubes one at the same time effects a uniform mixing of the dialysate. Particular care must be taken to avoid a source of error that often arises at this point, which is, that if the flask has been supplied

with too much toluol, or if the tube at the beginning of the test has not been immersed sufficiently deeply, then it may easily happen that, during the introduction of the pipette, some of the liquid passes from the outside into the dialysing tube. While, if one sucks strongly with the pipette at that moment, then the reverse may occur, and the contents of the tube may enter the pipette.

Ten cubic centimetres of the dialysate are taken, by means of a closed pipette passed through the toluol layer, and poured into a dry, wide, and absolutely clean test-tube. For each dialysate, as a matter of course, a separate, absolutely clean and dry pipette is used. One's work should never be arranged in such a manner that the pipette has to be hurriedly cleansed with alcohol, water, or ether after use, for the cleaning in this case may very easily be insufficient.

The danger of soiling the pipette with saliva is particularly great. (Compare p. 153.)

Then 0·2 c.c. of the 1 per cent. aqueous solution of ninhydrin are added to each test, together with a dry boiling rod (see p. 159), and one test after another is boiled absolutely evenly for a whole minute (see p. 160). After half an hour we ascertain which tests show a coloration and which do not, and only then do we compare our results with the original dialysates. If there be any tests which have evaporated more than the others, they are rejected, if they show a positive

reaction. It may sometimes happen that, for instance, the dialysate of the serum gives a negative reaction, while serum + organ shows a slight violet coloration. If both samples have been boiled equally according to the directions, then both will have evaporated equally, and in this case the slightest coloration may be considered as unconditionally positive.[17] If, on the contrary, the sample, organ + serum, has evaporated more, then we are confronted with the possibility that the stronger concentration is the cause of the coloration. In spite of the presence of absolutely equal quantities of substances, capable of reacting with ninhydrin, in the dialysate of the serum and that of the sample serum + organ, a higher concentration has been obtained owing to stronger evaporation. If it is impossible to effect even boiling by any other means, then it is necessary to resort to a water bath. The samples to be compared are placed in a stand, and immersed in a water bath. The boiling must be continued longer than when heating in an open flame, but two to three minutes are

[17] If the reaction is very weak, one may try to make it stronger in the following manner: To each of the cooled solutions—dialysate from the experiment serum alone, and serum + substrate—one again adds 0·2 c.c. of the ninhydrin solution, and boils for one minute. The reaction then often becomes stronger. Obviously we must in this case, too, make a comparison with the dialysate of the serum only. Our present experience is still too small to enable us to recommend this process for general use.

sufficient. As this method has not yet been applied to large quantities, the most suitable time has yet to be actually found.

Exact comparisons are only possible, when the test-tubes are of the same dimensions and have exactly the same thickness. For this purpose we must always arrange to have a sufficient stock of test-tubes answering perfectly to this requirement. In order to realize the importance of this proceeding, we only have to pour a slightly bluish solution into a wide and a narrow test-tube respectively, when we see that the former will show a much deeper blue colour than the latter. A faulty diagnosis would thus result.

The following cases are possible. The usual result of the reaction is either, dialysate of serum and of serum + organ negative, in which case no decomposition has taken place; and, if placenta had been used, we should assert that there was no placenta in the living state that had any connection with the particular organism; or else serum alone gives negative, and organ + serum positive, results. The diagnosis would indicate pregnancy, or, better still, the existence of a placenta that still stands in effective relations with the organism of the mother.

It may happen that the serum alone gives off substances to the dialysate, in sufficient quantity for the positive reaction to appear under the conditions

selected. If, in such a case, the sample of organ + serum shows a markedly stronger blue coloration, then the case has to be looked upon as positive in respect to the reaction. Should, however, the difference in the intensity of the coloration be very small, the experiment has to be performed again, using a less quantity, say 1 c.c., of serum. It would then be possible to ascertain whether decomposition had taken place or not, as the serum sample would be negative.

The appearance of the reaction should on no account ever be determined by artificial light. Again, it is not advisable to compare the test-tubes in their stands, but each one should be taken out separately, and examined against white paper by both transmitted and reflected light.

Breaches of this rule are very often committed. Many reactions are declared positive which, when thoroughly investigated, show not the slightest coloration. If a sample is marked as being just perceptibly positive, then a number of other samples should be changed about in the hand, and, only if the same sample can be unhesitatingly picked out as showing a coloration, should one's judgment concerning the reaction be relied on.

Difficulties are only experienced with reddish and yellowish-brown tones, but these have no relation whatever with the ninhydrin reaction. They can

easily be recognized by diluting a truly violet solution with water until the intensity of the colour corresponds with that of the sample, when one can see at once that, though the solution has been very much weakened, the colour still appears violet. A reddish, or, rather, yellowish-brown tint means that either the work has not been properly carried out, or else that the blood contained acids or alkalies in excess. The experiment must be repeated, otherwise it may happen that the existing conditions conceal a positive reaction. We shall deal with this point in fuller detail, when we return to the question of the sources of faulty observations.

Under certain conditions a special control test may be needed. Such would be the case in dealing with micro-organisms cultivated on a medium which could not be readily separated by centrifuging. In this case the germ-free medium must be boiled by itself, until the filtered boiled water gives no traces of coloration with ninhydrin. Then the cultures are prepared in exactly the same way, and the following tests are performed: (1) Serum alone; (2) serum + medium; and (3) serum + culture. Should the experiment (2) produce decomposition, then a positive reaction in experiment (3) would certainly not prove that the micro-organisms had been decomposed.

A very important control test, for proving the suitability of the organ or the substrate used, is the

following: About five to ten times more of the substrate than has been used for the test is taken together with 5 c.c. of water, and the whole is dialysed in an incubator for sixteen hours, against 20 c.c. of distilled water. Then the dialysate is evaporated on the water bath to 5 c.c., and the latter is boiled in the usual way with 1 c.c. of ninhydrin solution. The solution must remain absolutely colourless. According to my own experience this test always results negatively, when the substrates have been prepared in accordance with the directions. It is only necessary for the first testing of the organ, and is carried out if doubts arise as to the suitability of the latter. As the same organ is always used over and over again for experiments in which no decomposition is expected, we have a concurrent control over the suitability of the organ. Should errors occur in these experiments, then the dialysing tubes are immediately tested, as well as the organ, in the manner laid down. The statement that for the control experiment organ alone was used—0·5 gr. of the organ—and that 10 c.c. of the dialysate have given a negative result, always proves that the principles of the whole method have been misunderstood. An organ must have been very unsatisfactorily prepared, if the 20 c.c. of the dialysate contain such a quantity of substances reacting with ninhydrin that the reaction, after being conducted in the usual way, gives positive results.

We have described the performance of the experiment as it is carried out at the present time. Previously it was usual to make use of the biuret reaction for proofs of decomposition of albumen. To 10 c.c. of the dialysate 2·5 c.c. of a 33 per cent. solution of caustic soda were added, and this was then covered with a layer of very dilute copper sulphate solution. (See here p. 155.) If a violet to red ring appeared, the reaction was recorded as positive.

The biuret test has been entirely given up for the ninhydrin test, because the majority of observers have a difficulty in distinguishing with certainty a feeble biuret reaction. Those, however, who are capable of distinguishing a biuret reaction, however slight, should adhere to this test as well under all conditions.

Sources of Error in the Dialysation Process.

There are many possibilities leading to erroneous results. It is best to consider them from the point of view of utensils employed and manipulations adopted, and to refer again to the sources of error mentioned in the description of the method.

(1) *Tubes.*—We take it for granted that all tubes are thoroughly and accurately tested before anything else. On the average about 20 to 30 per cent. of the dialysing tubes supplied by the firm Schleicher and Schüll will be useless, because there are nearly

always some which allow the passage of albumen.[18] Or they may become useless subsequently, generally becoming permeable to albumen. This, however, only occurs when they are handled improperly. They must not be cleaned with a rough brush, nor must they be boiled for too long a time. Tubes may become impermeable to peptones through over-boiling, so that, though they should be thoroughly washed, they should be boiled but slightly. They must be kept in sterilized water with a thick layer of toluol (see p. 162), and must never be left for a long time unemptied of their contents.

A great source of error which is, however, impossible with proper manipulation, is due to tubes being insufficiently cleaned. The result of this is, that the wall of the tubes will contain traces of substances, which react with ninhydrin if sufficiently concentrated. They may be present in such minute quantities as to be unable of themselves to produce a coloration; yet they will, when added to the analogous substances that are present in the serum, convert a negative reaction into a positive one. Therefore the utmost possible care must be exercised in the manipulation of the tubes.

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[18] We have recently observed up to 80 per cent. of useless tubes. It would be very desirable if a dialysing tube could be produced which was, at the least, indubitably impermeable to albumen.

Tubes must be tested again about every four weeks. Should any error in diagnosis have occurred before this time, and other possible errors have been excluded, then the tubes must be immediately tested for permeability to albumen and for even permeability to peptones.

(2) *Serum.*—Here we have to deal only with its age, the possibility of an infection, hæmolysis, and the contents of the serum in respect of red blood corpuscles and of other form-elements. (See pp. 173-175.)

(3) *The Organ.*—This is nearly always the cause of errors in diagnosis. It is nearly always forgotten that, in the arrangement and execution of the experiment, we are dealing with quantitative conditions. Two cases have to be distinguished :—

(*a*) *The Biuret Reaction.*—The serum alone does not give off substances which diffuse and produce a biuret reaction ; so that, as regards compounds which give a biuret reaction, it must be reckoned as completely indifferent. It is comparatively easy to boil the organ in such a way, that the water in which it was boiled will not give any biuret reaction. If the ninhydrin reaction turns out negative, one can never obtain a biuret reaction. If such an organ be mixed with serum, and the dialysate now gives a positive biuret reaction, then we may be sure that decomposition has taken place. The conditions here are very simple.

(*b*) *The Ninhydrin Reaction.*—In order to understand the propositions that follow, we must be clear concerning the fact, that blood serum always contains, in varying quantities, substances which are to be found within the peptone group, and therefore react with ninhydrin. After a meal at which albumen has been taken, the quantity of such substances appearing in the serum immediately increases, in consequence of which the blood must be taken during a state of hunger.

A great many experiments have been necessary to determine what quantity of serum, in general, will give off to the dialysate only so much of the substances referred to, as is required for a negative reaction with ninhydrin. An insufficient quantity of serum must not be used, if the decomposition of the organ's albumen is to be as complete as possible. It has been found that, in general, 1·5 c.c. of the serum may be used. It is obvious that, under certain circumstances, an even greater quantity of serum may give off so few substances reacting with ninhydrin that the reaction of the dialysate still remains negative. Conversely it may happen that 1·5 c.c. of serum alone will give a positively reacting dialysate, which is the reason why a control test with serum alone is absolutely essential. The latter test indicates whether the serum in use answers the condition of not giving off, of itself, a sufficiency of substances to react with

ninhydrin. It is obvious, for the reasons mentioned, that exactly the same quantity of serum must be added to the organ, as has been used for the control test with serum alone. We must never, on the strength of the fact that the test with the serum alone gives a positive reaction, jump to the conclusion that, during the test, proteins have been decomposed in the serum. The substances producing this reaction were present from the beginning. If the reaction with serum alone turns out negative, then it simply means that the dialysate contains those compounds, which react with ninhydrin, in a state of concentration insufficient to produce a coloration; and this is the only conclusion we are entitled to draw from the result. It certainly does not indicate that there are no such compounds present. If one concentrates a dialysate of this kind, it eventually gives a positive reaction.

We therefore arrive at the fact, that we can only determine whether there are sufficient compounds present to give the coloration, but not what the quantities actually are. If, however, the following conditions are complied with, then this circumstance offers no difficulties. The organ must be absolutely free from substances, reacting with ninhydrin, which can be boiled out and so passed over to the filtrate. When the tubes are rinsed, no water should be allowed to enter them. The organ must be perfectly dried,

before the tube is filled with it. During storage in the incubator no evaporation must take place. Further, when boiling the actual samples, uneven ebullition must not be allowed. An example may help us to make these conditions clear. We will assume that twelve experiments have been made with serum obtained from non-pregnant individuals, and that the serum has in every case given negative results. We conclude that none of the dialysates have attained the necessary concentration in compounds that produce coloration with ninhydrin. Only from a certain concentration onwards is the coloration possible; and this limit we designate by the number 1. Then, to give an example, the cases quoted in the annexed table are possible:—

Case	Test with serum alone. Ninhydrin test.	Contents in the serum of compounds which, with ninhydrin at a sufficient concentration, react so as to produce a coloration	Test with organ+serum organ=0. Ninhydrin test	Test with organ+serum organ = 0.10 Ninhydrin test	Test with organ+serum organ = 0.50. Ninhydrin test
1	—	0·12	—	—	—
2	—	0·45	—	-	—
3	—	0·84	—	—	+
4	—	0·65	—	--	+
5	—	0·89	—	—	+
6	—	0·98	—	+	+
7	—	0·87	—	—	+
8	—	0·99	—	+	+
9	—	0·42	—	—	—
10	—	0·86	—	—	+
11	—	0·78	—	—	+
12	-	0·75	—	—	+

Three series of experiments were conducted with the same sera, and with equal quantities of these. In the first experiment the organ was + 0, *i.e.*, it was absolutely free from substances which could be boiled out and filtered, and which, under the strictest conditions, would produce a coloration with ninhydrin. In every case we had to add to the quantity of substances emanating from serum alone, and passed into the dialysate, 0 gr. of these compounds. Then, in the experiment serum + organ, the ninhydrin reaction obviously remains negative.

For the second experiment an organ was taken, which passed over to the boiled water just a trace of reacting substances. We will assume that it contained 0·10 gr.[19] of these compounds. This quantity is added to that which the serum gives off, and we have the positive reaction of Cases 6 and 8. The limital value, 1, has been exceeded. Thus, by means of a simple addition, a positive reaction has been obtained and, in consequence, two errors in diagnosis. The third column shows us how the ninhydrin reaction results, when we use an organ prepared in a still more imperfect manner.

Exactly the same position is reached, if the dialysate

[19] We take this here merely by way of an example. Obviously, in actual tests, the same quantity, *i.e.*, 0·10 gr., would never be transferred to the dialysate, if the organ can only give off that amount; some lesser quantity would pass over.

evaporates unevenly in the incubator. Take, for instance, Cases 6 and 8. In both cases the serum alone nearly reaches the limit, 1. Then, should the dialysate, in the experiment organ + serum, evaporate more strongly, or should the corresponding dialysate become more strongly concentrated, during boiling, than that of the relative control experiment, then we shall get a positive reaction owing, entirely, to the concentration; in which case we shall get a wrong result. These examples may be a warning to those who make use of a particular technique in an imperfect manner.

It is easy to understand that errors in diagnosis have often occurred, and that, on the other hand, brilliant results have been reported.

As a matter of fact our limit value, 1, is seldom attained. Unfortunately, this occurs just when carcinoma, myoma, salpingitis, exudates, suppurations, or hæmorrhages are present, that is, just when the method should diagnostically give the most valuable differential results. It is obvious that the investigation of such cases requires double care.

The performance, under absolutely equal conditions, of a particular experiment, and its control, is of decisive importance in regard to the results obtained. In the first place, absolutely pure distilled water must be used. Water, which gives an acid or alkaline reaction, leads inevitably to erroneous results. Ninhydrin

reacts not only with albumen and albuminous decomposites, but under certain conditions with other compounds as well, for instance, sugar.[20] No trouble can be caused by these, if distilled water be used. The organ cannot give off any non-albuminous substances, which will affect the reaction of the fluid, if it is boiled in the manner prescribed. There could not possibly be any carbohydrates left, and we have the control test with serum to fall back on, in any case. Were this to contain much sugar, and, in consequence, to interfere with the reaction of the outer fluid, then it is conceivable that a coloration might take place, which could not be referred to albuminous decomposites. This result, however, would appear in the test with serum alone, and also in the one with serum + substrate. Even the blood serum from cases of diabetes does not show any positive reaction ascribable to the presence of sugar. Non-compliance with the directions respecting water generally manifests itself in the fact, that a really positive reaction turns out negative; the reaction being, in fact, very sensitive towards acids and alkalies, *i.e.*, towards H and OH ions.

For the reasons laid down we must always boil the organs in distilled water, and preserve them, as

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[20] Vgl. W. Halle, E. Loewenstein und E. Pribram: "Bemerkungen über Farbreaktionen des Triketohydrindenhydrats (Ninhydrin)," *Biochem. Zeitschr.*, lv, 357. 1913.

well as the tubes, in this medium. The rinsing of the dialysing tubes must also be done with distilled water.

Finally, we must bear in mind another source of error, which we have not yet specially referred to. It may sometimes happen, that the substrate added to the serum absorbs some constituents of the latter, and retains them. Such a case would manifest itself in the fact, that the serum alone would react positively, while the dialysate of the experiment, organ + substrate, would give a negative reaction. Further, a reaction might give a negative result, although decomposition had actually taken place. The optical method would easily detect such sources of error.

There is no single point in the rules which lacks a definite foundation. Researches have generally been wrecked owing to trifling details. A glance at the literature, however, shows that at present the method is properly used in many places, and leads to surprisingly beautiful results.

Further sources of error are: The use of vessels that are not dry, and of boiling-sticks that have been touched by the hands, soiling the pipettes with saliva, inaccurate measurement of the ninhydrin solution, the use of infected water, the cultivation of bacteria in the same incubator as is used for experiments on the action of ferments, covering the contents of the tubes, and the outside fluid, with an

insufficient layer of toluol, changes of temperature in the incubator, and working in places where acid or alkaline vapours are developed.

These sources of error ought, properly, to occur very seldom.

On the other hand, the following point is often overlooked. After the tube has been filled with the organ, and the serum and the toluol have been added, it is absolutely necessary to make sure that the whole of the tissue is covered with the serum and toluol. Should the slightest portion of the tissue project above the toluol, it is then liable to decay in the course of sixteen hours, and so become a source of serious error.

In conclusion, we will add the following supplementary details, which are not at present in general use, because they are not considered as being absolutely necessary. We may, instead of the control with serum, use a control with organ + inactivated serum. The serum is heated for thirty minutes at a temperature of 60° C. This kind of control is capable of indicating an insufficiently prepared organ.

Starting with the idea that a certain limital value must be present, in order to give a colour reaction with ninhydrin, one might conclude that it would not suffice to test the filtered water, in which the organ was boiled, with 1 c.c. of ninhydrin solution. We have therefore produced a solution of silk-peptone,

which has been so strongly diluted, that 5 c.c. of the solution just fails to show any coloration with 1 c.c. of ninhydrin solution. 2·5 c.c. of this solution were then added to 2·5 c.c. of the filtrate obtained from the water in which the organ was boiled, and 2 c.c. of the ninhydrin solution were added to this. The mixture was boiled in the usual way for one minute, and the reaction remained negative. It would always have been possible for the limital value to be attained by means of additions. Further, a volume of 10 c.c. was reduced to 5 c.c. After the addition of 1 c.c., and later of 2 c.c. of the ninhydrin solution, no coloration appeared.

Finally, we may once more insist on the fact that an organ containing blood frequently fails to act, even when it fully complies with the conditions with reference to the water, in which the organ has been boiled (see pp. 164-168).

A desire has often been expressed, that we might have a special colour-scale for estimating the results of the ninhydrin reaction, with a view to recording the strengths of the reaction in a generally equivalent manner; but this cannot well be effected, because the ninhydrin reaction does not allow of sharp delimitation. With experience, each observer will soon be able to judge whether the reaction is strong, medium, slight, or very slight. Besides, we must not lay too much stress upon the intensity of the reaction. It is

quite possible, for instance, that in any given case a quantity of highly molecular peptones is present in the dialysate. The biuret reaction is surprisingly strong, while the ninhydrin reaction, on the contrary, is very weak. Conversely, we can imagine the extreme case, in which the decomposition lies below the peptone limit. We obtain a deep blue ninhydrin reaction, which means that many compounds, having the structure of amino-acids, are present; whilst the biuret reaction gives a negative result. These facts show clearly, that the ninhydrin reaction enables us to recognize many more compounds of the group of albumen decomposites than the biuret reaction.

Certainly many points in the whole method of the dialysation process might be modified. In the first place, the apparatus used could be improved. One might consider, for instance, the possibility of constructing an apparatus, which would enable us to boil the solutions of the ninhydrin reaction simultaneously and equally, and at the same time to prevent any evaporation. We have purposely made no propositions in this direction, because it seemed to us, that the great advantage of the present method is just that it is simple, clear and concise. We have also made experiments for simplifying the preparation of the organs, and more particularly for shortening that process. Studies on organs, that had been dried and pulverized at 37° C. with special precautions,

gave good results, but the risk of infection is great. In any case, organs prepared in this way have also to be tested each time before use. The boiling process has this advantage over the other, that the tissues are loosened, and in this way are more easily acted upon by the ferment.

II.—The Optical Method.

The principle of the Method.—The optical method enables us to demonstrate alterations in optically active substrates by a determination, with the aid of a polariscope, of changes in their angle of rotation.

The aim of the optical method is, in principle, exactly the same as that of the dialysation process. In the latter we determine the transformation of a colloid into a diffusible crystalloid. This transformation is the result of a hydrolytic decomposition. In the optical method we start, for purely technical reasons, not with albumen, but with peptone produced from the latter. We cannot use albumen, because it would prevent us determining the angle of deviation of the substrate-serum mixture. It would either give rise to precipitates, or else render the mixture so heterogeneous, that slight changes of rotation would be very difficult to follow. When using the optical method, we allow the decomposition, produced by the ferments present in the serum, to set

in later, than in the dialysation process. We remove part of the decomposition from the influence of the ferment, when we convert albumen into peptone in the test-tube. It must be our aim to maintain the peptone mixture in as high a molecular state as is possible, as experience has shown, that decomposites of too low molecularity are not attacked by some kinds of serum, which decompose more highly molecular peptones. It is very clearly shown, in this connection, that the conception of the unity of the proteolytic ferments does not correspond at all with the reality. There is not the slightest doubt, that different ferments exist for different stages of decomposition. The principal problem, in the application of the optical method to biological questions, was the elaboration of a method of dealing with highly molecular peptones, which are very closely related to the albumens.

The Application of the Optical Method.—This is very simple. 1 c.c. of serum, absolutely free from hæmoglobin, is placed in a test-tube. It must not contain any form-elements, and must be sterile. To this is added 1 c.c. of a 5 to 10 per cent. solution of peptone, prepared from the organ in question. Of course, peptones may also be prepared from bacilli, or else from certain proteins. The serum is mixed with the peptone solution and poured into a polarization tube, of a capacity of 2 c.c., and the angle of rotation of the mixture, at a temperature of

37° C., is determined. The deviations are noted at certain intervals. If there is no change in the deviation, then we conclude that no decomposition has taken place. Should there be an alteration in the rotation after some time, then we must infer a fermentative decomposition, such as has been demonstrated by special experiments with ferment solutions.

We shall now give a description of the preparation of the peptone.

Preparation of Peptones for use in the Optical Method.

Organs are first deprived of their blood, in exactly the same way as has been described on p. 164. They can then be subjected directly to hydrolysis, after the pieces of tissue have been dried, as much as possible, between filter papers. If it is desired to collect larger quantities of the same tissue, then the tissue, freed from blood, is boiled for ten minutes in water, and is subsequently preserved in sterilized water with chloroform and toluol. It is, of course, not necessary, in this case, to boil the organ to such an extent, as to deprive it of all substances reacting with ninhydrin. Boiling is merely resorted to here, in order to destroy any cell ferments that may be present; otherwise autolysis may manifest itself. As soon as enough of the organ has been collected,

then it is similarly freed from water, as far as possible, before being placed in sulphuric acid, which is kept cool by means of ice. Nervous tissue, after it has been deprived of all blood and boiled, must first be extracted with carbon tetachloride, as otherwise its lipoidal sheath makes decomposition very difficult. Tubercle bacilli must also be freed from lipoids.

For hydrolysis, we use 70 per cent. (by weight) of sulphuric acid, which must be cold. We take three times as much of this, as of the tissue to be decomposed. The vessel is energetically shaken, and then carefully stoppered. From time to time it is shaken again. The tissue is soon dissolved, the solution becoming more or less brown. After standing for exactly three days, at the temperature of the room (20° C. at most), the vessel containing the hydrolysate is placed into iced water, and diluted with ten times its quantity of distilled water. The addition must be made very gradually. The temperature of the solution is controlled by means of a thermometer, and must never be allowed to rise above 20° C. If the vessel is too small, then the solution is transferred into a larger one, and the water with which we are diluting is used to rinse the first vessel.

We now begin the neutralization of the sulphuric acid with barium hydroxide. Pure crystalline hydroxide is employed for this purpose, and this is gradually added, until the solution gives no

precipitate, either with barium hydroxide solution or with sulphuric acid. In the test with barium hydroxide it may happen, that a precipitate appears, even though no more sulphuric acid is present. These are barium salts of peptones, which separate out. They can be dissolved in nitric acid, while barium sulphate is insoluble in this.

Neutralization is carried out in such a way, as to calculate the quantity of barium hydroxide necessary, by the amount of sulphuric acid used. The barium hydroxide is best added in the solid form, and is well stirred until the action is complete. The neutralization of the sulphuric acid is first tested by means of litmus paper. Finally, small samples are filtered through a small funnel,[21] and then one sample is tested with barium hydroxide,[22] and another with sulphuric acid. If, in the first case, the solution becomes turbid, or precipitates are formed, then nitric acid is added, and the solution is slightly warmed.

[21] If there be a centrifuge at one's disposal, then we recommend centrifuging samples of the mixture. In this way a clear solution is obtained immediately without any loss of material.

[22] For testing purposes an aqueous solution of barium chloride gives better results than barium hydroxide, because the baryta water becomes turbid, owing to its affinity for carbonic acid, with consequent formation of barium carbonate. When using the above solution, the sample employed must never be returned to the original solution, but must be thrown away.

If the sediment remains, it is a sign that more barium hydroxide is to be added to the original solution. It is advisable, always to work with very dilute solutions of sulphuric acid and barium hydroxide, otherwise one may easily overshoot the mark.

When the solution is free from sulphuric acid and baryta, we proceed to filter it through a doubled sheet of folded filter paper, or, by means of a filter pump, through a hardened filter impregnated with animal charcoal. This process can be hastened by the use of a centrifuge. The precipitate of barium sulphate is stirred up with distilled water, well kneaded in a mortar with water, and then filtered again. It is advantageous, in order to ensure a good output of peptone, to repeat this washing out with cold water many times. The ninhydrin test can be applied at this stage, as a test of the satisfactory washing out of the precipitate. To a portion of the filtrate about 1 c.c. of ninhydrin is added, and the mixture is boiled for one minute. If the coloration is faint, or even negative, then the process of washing out is discontinued.

In the meantime, the process of concentration has been begun. As solutions of peptones produce a great deal of scum, the apparatus represented in fig. 9 is used. The latter allows the peptone solution to evaporate to dryness, at about 40° C., under highly reduced pressure. The drop funnel serves the

purpose of conducting the peptone solution, in drops, into the flask. These drops evaporate immediately, and no scum is formed.

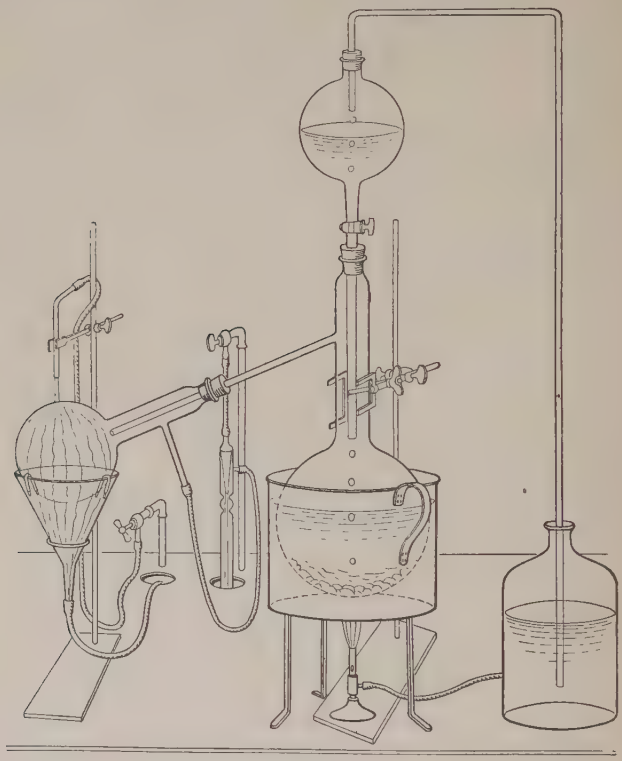

FIG. 9.

The peptone solution must never be strongly evaporated, until we have repeatedly satisfied ourselves, that it is actually free from sulphuric acid and

barium. With very dilute solutions traces of these compounds may escape detection. During the concentration of the solution, that of the sulphuric acid and barium hydroxide naturally increases, so that we may eventually get an hydrolysis of the peptone mixture.

Finally, we are left with a light yellow syrupy residuum. The latter is mixed with about 100 times its amount of methyl-alcohol, and the mixture is boiled. The boiling hot solution is filtered through a filter paper into about five times its amount of cold ethyl alcohol. It is well to put the latter into iced water. Precipitation is aided by the addition of ether. The whole is filtered, directly the precipitate begins to be formed. During the filtration, the filter should not be allowed to become empty. It is best to use a filter pump. Only at the end is the liquid allowed to pass entirely through the filter, after which the latter is immediately placed in a vacuum exsiccator. After a day or two the peptone is absolutely dry, and may then be weighed. First, a 10 per cent. solution, in 0·9 per cent. solution of common salt, is prepared, and the deviation of rotation of the solution is determined. If this is more than 1°, the solution is diluted, until it shows a rotation of about 0·75°. The higher degree of rotation would not be injurious. Dilution is only effected in order to make the best use of the costly material.

Standardization of the Peptone.—Let us assume that we have to deal with placenta peptone. This is mixed with the serum of individuals, who are certainly not pregnant, and then there should be no alteration of the original rotation. Should this not be the case, then the peptone is certainly not free from sulphuric acid or barium. With the serum of pregnant individuals a decomposition is bound to take place. At first, readings are taken every hour, and tests are made with many sera. A normal curve for the peptone is constructed, from the separate readings, by marking the angle of rotation on the abscissa and the time on the ordinate (*cf.* the curves given on pp. 62, 64, and 75-77). Once the normal alterations of rotation of the serum peptone mixture are known, then the readings for the diagnosis of normal cases need only be taken every four to six hours. If one has a special object in view, then the readings are taken more often.

The optical method supplements the dialysation process in many directions. In the first place, it is possible to determine quantitative differences in the speed of the decomposition. Further, qualitative differences may be observed. In the dialysation process, on the other hand, the dialysate may be used for experiments on animals and, for instance, be injected, in a state of concentration, for the purpose of deciding, whether certain

products of decomposition, contained in it, have a toxic effect.

To determine the range of rotation, a perfect instrument is necessary. The polarizing apparatus of Schmid and Hänsch, of Berlin, is one that answers

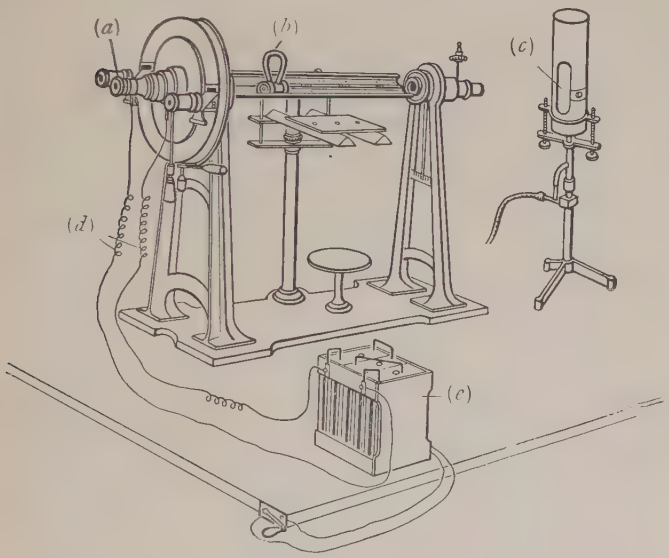

FIG. 10.

(*a*) Ocular for taking readings; (*b*) polarization tube; (*c*) sodium flame; (*d*) for illumination; (*e*) battery.

all the requirements (fig. 10). It allows of readings to the hundredth part of a degree. Since everyone makes individual errors in taking readings, *i.e.*, the range of rotation of the same solution is

differently observed, it has to be determined how great the limits of error are, on the average. It has been found, that most observers are capable of reading with accuracy to 0·02 of a degree. In order to attain greater certainty, we shall consider even a difference of 0·04 of a degree as the limit of error. Only with a change of rotation of 0·05 of a degree can decomposition be assumed to have taken place. The limit can thus be fixed without any danger, because, when an hydrolysis of the peptone does take place, the alteration of rotation is certainly more than 0·04 of a degree.

This method, as such, presents hardly any sources of error. At most, errors may be occasionally produced through turbidity, precipitates, and the like. Fortunately, however, in such cases, which actually very seldom happen with proper working, the reading of the rotation is impossible, and so this source of error disappears of itself. Of course, we should get no result if we were to try to polarize a cloudy solution.

A very important source of error would arise, if the range of rotation of the cold solution were taken for the initial value. The readings must be taken from the moment the contents of the tubes reach a temperature of 37° C. It is best to take the reading at the end of one hour, and take another at the end of the second hour. Values obtained in such a manner should, in general, not be too distant one

from the other, as decomposition begins, and manifests itself for a certainty, only after about six hours. Readings should not be followed up for more than thirty-six to thirty-eight hours.

Great progress would be made, if the reading of angles of rotation could be taken by means of some kind of automatic device. Objective values could be obtained, and we should be in a position to follow

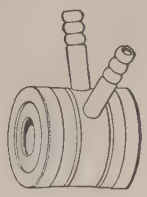

FIG. 11.

up details which, during the long intervals between readings, at present escape observation. Experiments in this direction, with the collaboration of Dr. Wildermuth, are in progress.

To obviate the cooling of the polariscopic tubes, during polarization, tubes have been constructed with water jackets (fig. 11). Lately, an electric heating apparatus[23] has been added to the polariscope itself.

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[23] Emil Abderhalden: "Ueber eine mit Polarisationsapparat kombinierte elektrisch heizbare Vorrichtung zur Ablesung und Beobachtung des Drehungsvermögens bei konstanter Temperatur. Zeitschr. f. physiol. Chem., 84, 300 (1913).

The former holds six polarization tubes, which can be brought into the field of observation without opening the heated incubator. In this way, all variations of temperature are avoided during the test. (See Plate.)

The most important source of error lies in the observer himself. The eye soon becomes tired, and it is impossible to take many readings at a time. One must become so experienced, as to be able to record a reading in at least thirty seconds. So soon as the eyes become weary, the readings become dubious. It is not advisable to resort to the optical method, until one has attained to sufficient certainty in taking readings.

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[1] Compare the most recent works, pp. 227-240.

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